Isolation and First Total Synthesis of PM050489 and PM060184, Two New Marine Anticancer Compounds

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ABSTRACT: Microtubules continue to be one of the most successful anticancer drug targets and a favourite hit for many naturally occurring molecules. Whilst two of the most successful representative agents in clinical use, the taxanes and the vinca alkaloids, come from terrestrial sources, the sea has also proven to be a rich source of new tubulin-binding molecules. We describe herein the first isolation, structural elucidation and total synthesis of two totally new polyketides isolated from the Madagascan sponge Lithoplocamia lithistoides. Both PM050489 and PM060184 show antimitotic properties in human tumor cells lines at sub-nanomolar concentrations and display distinct inhibition mechanism on microtubules. The development of an efficient synthetic procedure has solved the supply problem and, following pharmaceutical development, has allowed PM060184 to start clinical studies as a promising new drug for cancer treatment.

INTRODUCTION

The discovery and development of new drugs that can be used in the treatment of cancer remains one of the biggest challenges for the scientific community and the pharmaceutical industry. In recent decades there has been a boom in so-called targeted therapies and one of the most successful anticancer drug targets, tubulin, continues to be the focus of intense research efforts. Interestingly, nature has proven to be one of the best sources of new molecules that bind to tubulin and two of the most successful microtubule-targeting drugs, paclitaxel and the natural vinca alkaloids, were originally isolated from terrestrial sources.

The sea has also proven to be a highly productive source of compounds that bind to tubulin and some of them, such as discodermolide, dolastatins and hemiasterlin, have entered clinical trials but were later discontinued due to toxicity issues. More successfully, plinabulin, a synthetic analog of halimide, continues in clinical development (Figure 1) and eribulin (Halaven™), an analog of the marine natural macroclide halichondrin B synthesized by Kishi, was approved by the FDA and European authorities for breast cancer in 2010 and 2011, respectively.

As part of our continuing research programme to discover new anticancer compounds from marine sources, we describe herein the first isolation, full stereo-chemical structural determination, total synthesis and biological activity of two new marine natural products, PM050489 and PM060184 (1 and 2, Figure 2a), belonging to a new class of polyketides isolated from the sponge Lithoplocamia lithistoides collected in Madagascar and, to the best of our knowledge, hitherto uninvestigated. PM050489 and PM060184 have shown sub-nanomolar in vitro activity in human cancer cell lines, potent antimitotic activity, a new biochemical mechanism of interaction with tubulin and potent in vivo activity in different animal models, thereby demonstrating that tubulin continues to be a valid target for cancer treatment. Based on its activity, distinct mechanism of action and good safety profile, PM060184 has entered clinical development and Phase I trials are currently underway in France, Spain and the United States of America.

Figure 1. Chemical structures of tubulin binders. Marine natural products: (a) Discodermolide, (b) Hemiasterlin, (c) Dolastatin 10. Synthetic compounds inspired by marine natural compounds: (d) Plinabulin, (e) Eribulin mesylate.
RESULTS AND DISCUSSION

Isolation and structural elucidation. PM050489 (1) and PM060184 (2) were isolated from extracts (CH<sub>3</sub>Cl<sub>2</sub>:MeOH, 50:50) of the sponge Lithoplocamia lithistoides and purified by reverse-phase RP-18 chromatography and semipreparative HPLC. Initially, 1.6 mg (0.003%) of PM050489 (1) was obtained from a 61 g sample of frozen L. lithistoides. Isolation of further 1 from a second larger group of sponge samples yielded 160.8 mg of 1 (0.002%) and 2.6 mg of 2 (0.00003%) from 7.66 Kg of frozen animal material. The positive ion high-resolution (ESI) mass spectrum of 1 displayed a pseudomolecular ion at m/z 606.2940 [M+H]<sup>+</sup> with an isotopic distribution consistent with the presence of a chlorine atom in the molecule. These MS data and the presence of 31 signals in its <sup>13</sup>C NMR spectrum (Table 1) gave a molecular formula of C<sub>31</sub>H<sub>44</sub>ClN<sub>3</sub>O<sub>7</sub> for the compound (calcd. for C<sub>31</sub>H<sub>45</sub>ClN<sub>3</sub>O<sub>7</sub>, 606.2946).

The analysis of the HSQC spectrum revealed the presence in the molecule of six aliphatic and one oxygenated methyl groups, three aliphatic methylene units, and 13 methines, including one aliphatic, one nitrogemined, two oxygenated, and nine olefinic. In addition, the <sup>1</sup>H spectrum displayed signals for eight quaternary carbons, attributed to the presence in the molecule of three carbonyl groups, one carboxylate, three quaternary olefinic, and one quaternary aliphatic carbon. Two signals in the <sup>1</sup>H spectrum at δH 8.78 and 6.51 ppm did not display any correlations in the HSQC spectrum and were therefore assigned to two NH amide groups based on COSY and HMBC correlations.

Careful study of the COSY and HMBC spectra of 1 led to the identification of four discrete spin systems (A-D) represented in Figure 2b (H3-H28, H9-H12, NH14-H15 and NH17-H25). Connectivity between these different systems was established by <sup>1</sup>H-<sup>13</sup>C long range correlations observed from HMBC experiments. Indeed, cross peaks between Me28 and C7, C8 and C9, H9 and C7, and from H7 and H10 to C8 linked segments A and B. Likewise, correlations from H11, H12, NH14 and H15 to C13 coupled segment B to C through an amide carbonyl group (C13). Finally, segments C and D were linked in a similar manner based on the observation of HMBC correlations from H15 and NH17 to C16.

The presence of a tert-butyl group in the molecule was inferred from the presence of a single signal at δH 1.04 ppm in the 'H NMR spectrum integrating for nine protons, which correlated in both the HSQC and HMBC spectra with an intense <sup>13</sup>C signal at δC 26.7 ppm accounting for three of the carbons in the molecule. Other HMBC correlations observed between the methyl groups Me30, Me31, and Me32 and carbons C15 and C29, and from H15 to carbons C29 to C32 showed the presence of a tert-leucine (tert-butylglycine) in the molecule and confirmed the location of this amino acid residue in the structure of 1. Additionally, the presence of a 6-substituted 3-methoxy-5,6-dihydro-2H-pyran-2-one in PM050489 (1) was shown by cross-peaks in the HMBC spectrum from H3, both H4 protons, and the oxygenated methyl group Me26 to C2, and from H3 and H5 (weak) to the carbonyl group C1.

A signal in the <sup>13</sup>C NMR spectrum at δC 157.2 ppm accounted for the presence in the molecule of a carbamate group that was placed at C21 on the basis of a 1H-<sup>13</sup>C long range correlation observed between H21 and C33 in the HMBC spectrum. The two NH protons of this latter group appeared in the 'H NMR spectrum as a broad signal that changed intensity and chemical shift depending on the temperature and concentration of the sample. Finally, to complete the planar structure of 1, the chlorine atom present in the molecule was placed at C24 in agreement with the substitution pattern observed for the C23-C24 double bond and the chemical shifts measured for these two olefinic carbons.  

The geometry of the double bonds in 1 was determined on the basis of NOESY correlations (Figure 2b) and <sup>3</sup>H-H coupling constant values. Values of <sup>3</sup>J<sub>H2-H3</sub> = 11.6 Hz, <sup>3</sup>J<sub>H2-H14</sub> = 11.6 Hz, and <sup>3</sup>J<sub>H18-H19</sub> = 9.7 Hz, and NOESY correlations between the pairs H9/H10, H11/H12, and H18/H19 gave a Z geometry for the double bond at C9-C10, C11-C12, and C23-C24. On the other hand, the E geometry of the olefins at C2-C3, C7-C8, and C23-C24 was established through NOESY cross peaks observed between Me26/H3, H6/Me28, and H22/Me25, respectively. Additionally, the E geometry of the C7-C8 olefin was also supported by a NOESY correlation between H7 and H9.

To determine the absolute configuration at C15, the Marfey’s method for chiral amino acid analysis was used. Compound 1 was dissolved in 6 N HCl and heated at 110 ºC for 16 hours in a sealed vial. After evaporation of the solvent under a N2 stream and reaction of the residue with L-FDAA (Nα-(2,4-dinitro-5-fluorophenyl)-L-alanamide), the mixture was subjected to reversed phase HPLC-MS analysis. Comparison of the retention time of the derivatized tert-leucine with that of the derivatized R and S standards of the amino acid...
established the S configuration for the chiral center at C15 in PM050489 (1).

Table 1. $^1$H and $^{13}$C NMR (CDCl$_3$, 500 and 125 MHz) assignments for PM050489 (1) and PM060184 (2).  

<table>
<thead>
<tr>
<th>Position</th>
<th>$\delta_C$, mult.</th>
<th>$\delta_H$ (m, $J$ in Hz)</th>
<th>$\delta_C$, mult.</th>
<th>$\delta_H$ (m, $J$ in Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>161.6 C</td>
<td>-</td>
<td>161.6 C</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>145.2 C</td>
<td>-</td>
<td>145.2 C</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>108.2 CH</td>
<td>5.63 (dd, 6.5, 2.6)</td>
<td>108.2 CH</td>
<td>5.63 (dd, 6.6, 2.7)</td>
</tr>
<tr>
<td>4</td>
<td>26.1 CH$_2$</td>
<td>2.45 (ddd, 17.3, 11.5, 2.6)</td>
<td>26.1 CH$_2$</td>
<td>2.44 (m)</td>
</tr>
<tr>
<td>5</td>
<td>81.9 CH</td>
<td>4.23 (dd, 11.5, 7.1, 4.1)</td>
<td>81.9 CH</td>
<td>4.25 (dd, 11.3, 7.0, 4.0)</td>
</tr>
<tr>
<td>6</td>
<td>37.1 CH</td>
<td>2.85 (ddq, 9.9, 7.0, 6.7)</td>
<td>37.1 CH</td>
<td>2.85 (ddq, 9.9, 7.0, 6.7)</td>
</tr>
</tbody>
</table>

The $S$ absolute stereochemistry at C5 was elucidated by Mosher analysis of the corresponding hydroxyl group, obtained after reduction of the lactone and protection of the resulting primary hydroxyl group at C1 as its TBS (tert-butyldimethylsilyl) ether (Scheme 1). Treatment of 1 with NaBH$_4$ (Sodium borohydride) yielded 1,5-diol 3 that was regioselectively converted into its 1-TBS derivative 4 by reaction with TBSCl (tert-butyldimethylsilyl chloride). Esterification of this compound with $S$- and $R$-MTPA-Cl ($\alpha$-methoxy-$\alpha$-trifluoromethylphenylacetyl chloride) yielded compounds 5a and 5b in which elimination of the C26 methyl group and conversion of the resulting enol to the ketone had taken place in addition to the formation of the corresponding $R$- or $S$-MTPA ester at C5. Analysis of the $\Delta \delta (R,S)$ values obtained for each derivatives (Figure 2c) indicated the S absolute stereochemistry for the C5 chiral center according to Riguera's predictive models for MTPA esters.7
In the case of PM060184 (2), the positive ion high-resolution (ESI) mass spectrum displayed a pseudomolecular ion at m/z 594.3512 [M+Na]^+ with an isotopic distribution consistent with the absence of a chlorine atom in the molecule. These MS data and the presence of 31 signals in its 1^H NMR spectrum (Table 1) gave a molecular formula of C_{39}H_{48}N_{3}O_{7}Na for the compound (calcd. for C_{39}H_{48}N_{3}O_{7}Na 594.3510). The 1^H NMR spectra of 2 were identical to the corresponding spectra for 1 except for the presence of an extra olefinic proton (at 5.60 ppm), which was assigned to the C24 position by COSY and HMBC. The geometry of the C23-C24 olefin was assigned as Z for 1 for the presence of an extra olefinic proton (at 5.60 ppm), which was assigned to the C24 position by COSY and HMBC. The geometry of the double bonds C2-C3, C7-C8, C9-C10 via Wittig olefination, and Evans oxazolidinone-mediated aldol condensation between aldehyde C and (R)-4-Benzyl-3-propionyl-2-oxazolidinone to control the C5 and C6 carbon centers. For fragment B, the C21 stereocenter is introduced through resolution of racemic epoxide D, addition of propyne, and regioselective hydrozirconation followed by Cl substitution with N-chlorosuccinimide or by catalytic hydrogenation.

**Figure 3.** Retrosynthetic analysis of PM050489 (1) and PM060184 (2).

**Synthesis of fragment A.** The starting point for the implementation of this strategy was the alcohol 8 (Scheme 2) obtained in six steps from 1,3-propanediol and (R)-4-benzyl-3-propionyl-2-oxazolidinone. Alcohol 8 was oxidized to aldehyde 9 in 90% yield using sulfur trioxide pyridine complex. Wittig reaction of aldehyde 9 with the stabilized ylide (1-Ethoxycarboxylethylidene)triphosphorane afforded stereoselectively (EZ=95:5) the corresponding ester 10, which was reduced with DIBAL (diisobutylaluminum hydride) to allylic alcohol 11 in 77% yield. To introduce the diene moiety, alcohol 11 was oxidized with manganese(IV) oxide to aldehyde 12, which was then converted to vinyl iodide 13 by olefination with (iodomethyl)triphenylphosphonium iodide in 84% yield with a 95:5 ratio of Z:E isomers. Homologation of 13 began with regioselective deprotection of the primary TBS ether using PPTS (pyridinium p-toluenesulfonate) in EtOH to give alcohol 14 in excellent yield (93%) before oxidation to aldehyde 15 with sulfur trioxide pyridine complex in 80% yield. Horner-Wadsworth-Emmons olefimation of aldehyde 15 with diethyl (meth-
oxy(methoxycarbonyl)methylphosphonate\textsuperscript{a} provided ester 16 as a 90:10 mixture of (E,Z) isomers. On treatment of the isomers with HCl in MeOH, only the major E-isomer was able to cyclise, giving the desired lactone 17 (5S,6S, relative anti configuration) in a yield of 94%. Lactone 17 serves as fragment A for the synthesis of both 1 and 2.

Scheme 2. Synthesis of Fragment A.\textsuperscript{a}

\begin{align*}
\text{Scheme 2. Synthesis of Fragment A.} \quad & \text{a) SO}_3\text{Py, EtO}, \text{CH}_2\text{Cl}_2-\text{DMSO (2.2:1), 0 °C, 2 h, 90%} \quad \text{b) carboethoxyethylidene-triphenylphosphorane, toluene, 60 °C, 17 h, 96%} \quad \text{c) DIBAL, THF, -78 °C, 1.5 h, 59% (diastereomer 23)} \quad \text{d) MnO, EtO, 23 °C, 25 h, 93%} \quad \text{e) iodomethyl triphenylphosphonium iodide, NaHMDS, DMPU, THF, -78 °C, 2 h, 84%} \quad \text{f) PPTS, EtOH, 23 °C, 45 min, 95%} \quad \text{g) diethyl(methoxy(methoxycarbonyl)methyl)phosphonate, KHMDS, 18-crown-6, THF, -78 °C, 1.5 h, 59% (16) and 85% (diastereomer 24)} \quad \text{h) diethyl(methoxy(methoxycarbonyl)methyl)phosphonate, KHMS, 18-crown-6, THF, -78 °C, 1.5 h, 59% (16) and 85% (diastereomer 24)} \quad \text{i) HCl 37%, MeOH, 23 °C, 6 h, 94% (17) and 57% (diastereomer 24)} \quad \text{j) (i) pivaloyl chloride, EtO, THF, 23 °C, 17 h; (ii) HCl 37%, MeOH, 23 °C, 3 h, 33%; (k) Dess-Martin periodinane, CH2Cl2, 23 °C, 45 min, 95%; (l) NaBH4, MeOH, -78 °C, 40 min, 80%; (m) TBSOTf, 2,6-lutidine, CH2Cl2, 23 °C, 30 min, 95%; (n) (i) NaOH, MeOH, 40 °C, 4 h, 91%; (ii) Dess-Martin periodinane, CH2Cl2, 23 °C, 1 h, 91%}
\end{align*}

In order to complete the full stereochemical elucidation of 1 and 2, we also synthesized the (5R,6S)-lactone (relative syn configuration), from intermediate 14 following the strategy of oxidation/reduction of the alcohol at C5. Orthogonal protection of alcohol 14 with pivaloyl chloride and removal of the TBS ether gave intermediate 18. Oxidation of 18 with DMP (Dess-Martin periodinane)\textsuperscript{3} afforded ketone 19 which was nonselectively reduced with NaBH4 to obtain a mixture of diastereoisomers (R,S)-20 and (S,S)-18 which were separated by column chromatography. Protection of the secondary alcohol of 20 as a TBS ether with TBSOTf ( tert-butylidimethylsilyl trifluoromethanesulfonate) afforded compound 21. Finally, deprotection of the pivaloyl ether with NaOH and oxidation of the resulting primary alcohol with DMP yielded aldehyde 22 which was transformed into lactone 24 (5R,6S, relative syn configuration) via ester 23 as described previously. Instead of using J-based configurational\textsuperscript{4} analysis to determine the configuration at C6, we compared the NMR spectroscopic data of lactones 17 (5S,6S) and 24 (5R,6S) and observed multiplicity patterns for protons H5 and H6 of the anti lactone 17 that were very similar to those of 1, whilst great differences were observed in the case of the syn lactone 24 (Figure 4). As such, the syn configurations (5R,6S) and (5S,6R) could be eliminated leaving only the two anti configurations, (5S,6S) and (5R,6R), as remaining possibilities. Then, since the absolute configuration at C5 had already been unambiguously assigned as S (see above), the C6 stereochemistry could also now be unambiguously assigned as S.

Figure 4. Lactones 17 and 24.
Synthesis of fragment B. Having established efficient access to the common lactone-diene framework of 1 and 2, the next challenge consisted in the synthesis of fragment B. Our starting material was but-3-en-1-yloxy-tert-butyldimethylsilane (Scheme 3). The required chiral center at C21 was obtained from racemic butene oxide (±)-25 by way of Jacobsen’s hydrolytic kinetic resolution. Addition of a lithium anion derived from propyne to (R)-26 in THF then yielded alcohol 27, and subsequent conversion to the PMB (p-methoxybenzyl) ether provided 28. Gratifyingly, when chiral alkyne 28 was reacted with three equivalents of Schwartz’s reagent (Cp2ZrHCl, bis(cyclopentadienyl)zirconium(IV) chloride hydride) in toluene at 50 ºC, followed by addition of N-chlorosuccinimide, 29 was obtained in high yield and with excellent control of the olefin geometry (90% yield, 100% regioselectivity). PMB proved to be a key group in the hydrozirconation of alkyne 28 with the TBS or TBDPS (tert-butyldiphenylsilyl) ether analogs of 28 being found to give low levels of regioselectivity with Schwartz’s reagent and mixtures of the (E)-2-chloroalkene and (E)-3-chloroalkene isomers. Cleavage of the TBS group of 29 with TBAF (tetra-n-butylammonium fluoride) solution in THF, afforded alcohol 30 which was converted to the vinyl iodide 32 using similar methodology as employed in fragment A. The most favorable oxidation process for alcohol 30 were Piancatelli conditions, employing BAIB ((diacetoxyiodo)benzene) and a catalytic amount of TEMPO (2,2,6,6-Tetramethylpiperidin-1-yl)oxyl.

After several attempts to use the PMB ether throughout the synthetic sequence, problems with the final deprotection of the PMB ether forced us to abandon this intermediate in favour of the TBS analogue 34. The PMB protecting group of 32 was removed with DDQ (2,3-dichloro-5,6-dicyano-1,4-benzoquinone) in aqueous CH3Cl18 and purification of 33 simplified by treating the mixture with NaBH4 to reduce the p-methoxy-benzaldehyde by-product to the alcohol. Protection of secondary alcohol 33 using TBSOTf and 2,6-lutidine was straightforward and gave the key vinyl iodide 34 required for the synthesis of 1.

For the synthesis of 2, we used alcohol 27 as starting material to prepare vinyl iodide 39 which is an analog of 34 but without chlorine. In this case, we decided to use TBDPS as protecting group and reaction of 27 with TBDPSCl (tert-butyldiphenylsilyl chloride) in the presence of DMAP (4-dimethylaminopyridine) afforded intermediate 35 which was subjected to Lindlar catalytic hydrogenation19 at atmospheric pressure to obtain stereoselectively Z alkene 36. Finally, vinyl iodide 39 was obtained via intermediates 37 and 38 using the same synthetic strategy as used for 32.

With vinyl iodides 34 and 39 in hand, we needed to introduce the final chiral part of fragment B. Boc-tBu-Gly-NH2 (N-(tert-butoxycarbonyl)-L-tert-leucine-amide), prepared following Pozdnev procedure,20 was reacted with 34 and 39 under Buchwald conditions,21 affording enamides 40 and 41 in moderate yield (44-53%). The propensity of both the TBS and TBDPS protecting groups to cleavage under acidic conditions forced us to remove the Boc group by pyrolysis. Dissolution of 40 and 41 in ethyenglycol and heating at 200 ºC for not more than 20 minutes afforded amines 42 and 43 with longer reaction times giving degradation.

The reagents HOAt (1-hydroxy-7-azabenzotriazole) and HATU (N,N,N’,N’-Tetramethyl-O-(7-azabenzotriazol-1-yl)uronium hexafluorophosphate) are widely used in peptide synthesis to give rapid couplings with minimal loss of chiral integrity.22 As such, these reagents were used to couple amines 42 and 43 to the carboxylic acid 44 affording stannanes 45 and 46 in 66-77% yield. Carboxylic acid 44 was prepared in two steps by hydrostannation of ethyl propiolate with tributyltin hydride followed by hydrolysis with aqueous LiOH in THF.
Scheme 3. Synthesis of Fragment B.\(^a\)

\[ \text{Scheme 3: Synthesis of Fragment B.} \]

**Final steps.** At this point, the fragment A (lactone 17) and fragment B (vinyl stannanes 45 and 46) intermediates required to make 1 and 2 were now all available. For the final coupling, use of CuTc (copper(I) thiophene-2-carboxylate) in NMP (1-methyl-2-pyrrolidinone) under Liebeskind conditions\(^b\) provided trienes 47 and 48 in 66% yield (Scheme 4). Cleavage of the TBS or TBDPS groups with TBAF in THF generated compounds 49 and 50 in adequate yields. Introduction of the C21 carbamate moiety was then achieved by reaction of 49 and 50 with trichloroacetyl isocyanate and subsequent treatment with neutral alumina finally yielded the desired compounds 1 and 2 in 81-96% yield. All the spectral data (\(^1\)H & \(^{13}\)C NMR, optical rotation, IR, etc), HPLC retention times and biological activities of the synthetic samples exactly matched those of the isolated natural products.

**Scheme 4. Final steps of the synthesis of PM050489 (1) and PM060184 (2).\(^a\)**

\[ \text{Scheme 4: Final steps of the synthesis of PM050489 (1) and PM060184 (2).} \]

Following completion of the total synthesis of 1 as described above, the R absolute configuration could finally be assigned to C21. This was achieved by using racemic epoxide (\(\pm\))-25 to prepare intermediate 34 as a mixture of epi-
mers and subsequent downstream conversion to obtain as a mixture of the two alcohol epimers at C21 with (5S,6S,15S,21R) and (5S,6S,15S,21S) stereochemistry. The two epimers were separated by HPLC and the stereochemistry of each assigned by preparing the Mosher derivatives, before the introduction in each derivative the carbamate moiety. The NMR spectra of the natural product clearly matched those of the 21-R epimer whereas major differences were observed for the 1H and 13C resonances in the region around the C21 chiral center in the 21-S stereoisomer thereby confirming the R configuration for C21 in 1. In the case of 2, the lack of chlorine at C22 means that although there is no change in the spatial disposition of the different groups around C21, the descriptor of the C21 configuration is now S and that of the double link C23-C24 is now E (not Z as in 1) due to the different assignment of priorities according to the CIP (Cahn-Ingold-Prelog) priority rules.

In summary, the first total synthesis of PM050489 (1) and PM06084 (2) was achieved in a total of 35 and 33 steps, respectively. 18 steps from 1,3-propanediol is the longest linear sequence required to synthesize these marine natural products. The highly convergent synthesis we report herein has been industrialized and has also been used for the synthesis of new analogues.

**Biological evaluation.** Two bioassays were conducted in parallel during fractionation of the sponge extracts: (i) cell killing ability, evaluated against a panel of three human tumor cell lines, including colon (HT-29), lung (A-549), and breast (MDA-MB-231), and (ii) antimitotic activity, measured using a specific microplate immunoassay (ELISA). The in vitro antiproliferative activity was assessed using the colorimetric SRB (sulforhodamine B) method, performed as described previously. The GI50 (nM), concentration that causes 50% growth inhibition, values obtained for PM050489 (1) and PM06084 (2) in this assay were: 0.46 and 0.42 (HT-29), 0.38 and 0.59 (A-549), and 0.45 and 0.61 (MDA-MB-231), respectively. No selectivity was observed between cell lines. The antimitotic activity was assessed in a modified cell-based immunoassay using the specific mitotic marker MP-2, an epitope found in a set of phosphoproteins that are specifically phosphorylated during mitosis. PM050489 (1) displayed an IC50 (compound concentration that produces 50% of mitotic arrest in the cell population) of 26.4 nM (0.016 μg/mL) when tested under these conditions.

**CONCLUSION**

We have achieved the first isolation, structural elucidation and total synthesis of PM050489 (1) and PM06084 (2), the first members of an unprecedented new class of polyketides isolated from extracts of the Madagascan sponge *Lithoplocamia lithistoides*, with sub-nanomolar in vitro activity in human tumor cell lines, potent antimitotic properties and a distinct inhibition mechanism on microtubules.

The development of an elegant and efficient total synthesis at multigram scale has provided a solution to the supply problem, and together with pharmaceutical development work, mechanism of action and preclinical studies, has allowed us to initiate the clinical development of PM06084 (2) as a promising drug for cancer treatment.

**REFERENCES**


