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The Design and Synthesis of Guanosine Compounds with In Vitro Activity against the Colon Cancer Cell Line SW480: Non-Taxane Derived Mimics of Taxol?

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Dedicated to Professor David Crout to mark the occasion of his retirement.

Abstract—In the course of our investigation into the use of taxol as a lead compound to design new molecules with anti-cancer activity, we have synthesized four compounds based on protected guanosine coupled to taxol isoserine side-chain analogues. These analogues show in vitro anti-cancer activity against the colon cancer cell line SW480 that their constituent parts do not.

It has been known for many years that the leaves and berries of the yew tree are toxic to animals and man. The constituent chemicals that cause the toxicity are collectively known as taxanes. Some of the more complex naturally occurring taxanes, taxol **1** and cephalomannine **2**, show potent anti-leukemic and anti-tumor inhibitory and therapeutic properties.[1]

A problem arises when a large amount of taxol is required. Taxol originates from the bark of the yew tree and hence its extraction ultimately kills the tree. This has severely damaged the population of one of the slowest growing trees in the world. The problem has been partially circumvented by semi-synthesis methods that utilise similar compounds to taxol, found in the needles of the yew tree, these compounds are then converted into taxol. Although this produces a renewable source of taxol, the method allows for little modification of the taxol skeleton to produce more active anti-cancer drugs, and results in an extremely expensive treatment for cancer.

An alternative, the total synthesis of taxol, has been achieved.[2] Over the last two decades, an incredible amount of time and money has been directed towards this goal.[3] However, any total synthesis of taxol to date involves over 35 steps, and it is highly unlikely that any alternative synthesis will involve any less than this number, due to the complexity of taxol. With this number of synthetic steps, a total synthesis of taxol is probably not a commercially viable procedure at this moment.

All current bioactive analogues of taxol result from modifications of the functional groups on the taxane skeleton, or modification of the size of the carbon rings contained within the molecule. Again, in general, taxol itself is the starting point for such modifications.

The challenge would therefore appear to be to produce a synthetic non-taxane compound, that mimics taxol, or more precisely, mimics the molecule that taxol takes on the role of or interferes with during cell division. This non-taxane taxol mimic should have equivalent, or better, chemotherapeutic properties as taxol. Furthermore, this synthetic compound should be predisposed to modification to produce specific compounds for the treatment of specific cancers. Finally, it would be extremely beneficial if these new compounds could be producible in a commercially viable manner.

Given the enormity of the challenge presented above and the associated problems, the logical starting point is to use taxol as a lead compound. An overall assessment of previous investigations into the functionality in the

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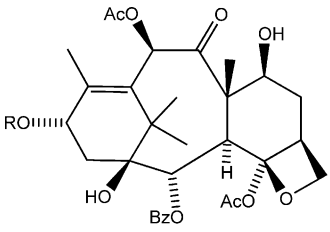
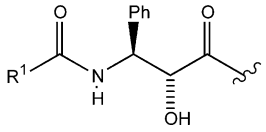
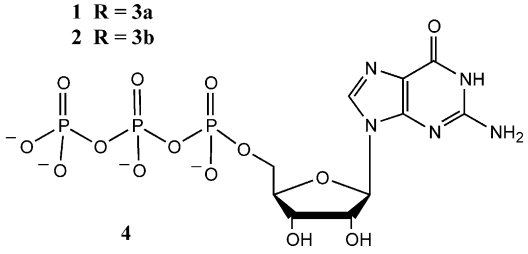


1 R = 3a
2 R = 3b



3a R^1 = Ph
3b R^1 = C(Me)=CHMe



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taxol molecule which lead to its activity as an anti-cancer compound, and of various other biological observations should produce a hypothesis as to mechanism through which taxol attains its bioactivity. In other words, we must speculate as to with which biomolecule taxol has a biosteric and bioelectronic relationship.

Amongst the many cellular functions dependent on microtublules is the process of cell division. Microtubule polymerisation from tubulin is disrupted by the presence of taxol, through its ability to reduce the critical concentration of tubulin required for spontaneous assembly. In turn, this leads to cell death.[4] Experiments show that the activity of taxol is dependent on several conditions. The basic carbon skeleton of taxol must remain intact, modifications of ring sizes and ring junction stereochemistry have generally resulted in analogues with reduced activity. There must be an isoserine side-chain present. If the side-chain is not present, there is no anti-cancer activity.[5] The isoserine side-chain must contain a free hydroxyl group. Any modification of this hydroxyl group, for example conversion to an ether, will greatly reduce the activity of taxol. All other modifications to the functionality around the taxol skeleton serve only to vary the activity by a varying degrees.[6]

Other experiments[7] have been carried out which reveal some interesting connections between taxol and its possible mode of action. Cells will normally only divide when guanosine triphosphate **4** (GTP) is present. Cells with no GTP present, but with taxol present, will commence division and then stop part way through the process. Removal of the taxol from the cells and replacement of GTP will restart cell division. These three observations clearly indicate that there is a connection between taxol and GTP.[8]

There are other facts which support this connection.[10] The tubulin dimer binds two molecules of GTP when involved in the production of microtubules, which are essential to cell division.[11] One of the GTP binding sites is on the tubulin,[4,9] the other GTP binding site's whereabouts is not known. Only GTP bound tubulin allows polymerization or growth of microtubules and the known tubulin GTP binding site is not competed for by taxol. It is thought that taxol binds to the tubulin and there is no evidence for or against both GTP binding sites being on the tubulin.

It is known that GTP is always bound to magnesium in vivo. This binding occurs through two of the oxygen anions. The binding of magnesium by GTP is essential to its function in the energy transfer process.[7] Perhaps this is the reason why it is necessary for the free hydroxyl to be present in the isoserine side-chain. The hydroxyl group would bind strongly to magnesium, and the amide nitrogen would also bind to magnesium, but the binding would be much weaker. Removal or conversion of the hydroxyl group into other functionality would greatly reduce the side-chain's capacity to bind to magnesium.

The molecular modelling studies[10] that we have carried out have shown that there is a similarity in the size, shape, electron density and many possibly important intramolecular distances between taxol and GTP. Some of the similarities are obvious, such as shape and distribution of electronegative atoms.

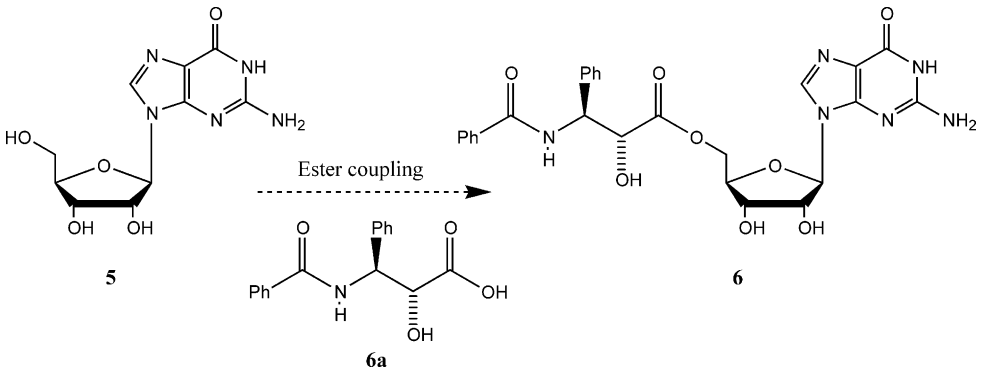
Our required and initial hypothesis is therefore that *taxol acts as a GTP mimic*. The basic carbon skeleton of taxol, with its incumbent functionality, acts as the guanosine section of GTP, and to a degree the isoserine side-chain acts similarly the triphosphate chain of GTP. This hypothesis is perhaps further supported when we study other molecules that have the ability, like taxol, to stabilize microtubules, although they are totally different in chemical structure terms. These molecules are epothilone,[11] sarcodictyin,[12] and eleutherobin.[13] Molecular modelling of these compounds clearly shows their similarity to taxol and GTP in their shape and electronic potential distribution.[10]

If the hypothesis stated above is in any way correct then there is a direct and efficient method for achieving our challenge stated earlier. We have only to take the side-chain **6a** and link it to guanosine **5** to produce hybrid **6** (Scheme 1). Molecule **6** should have the same or similar

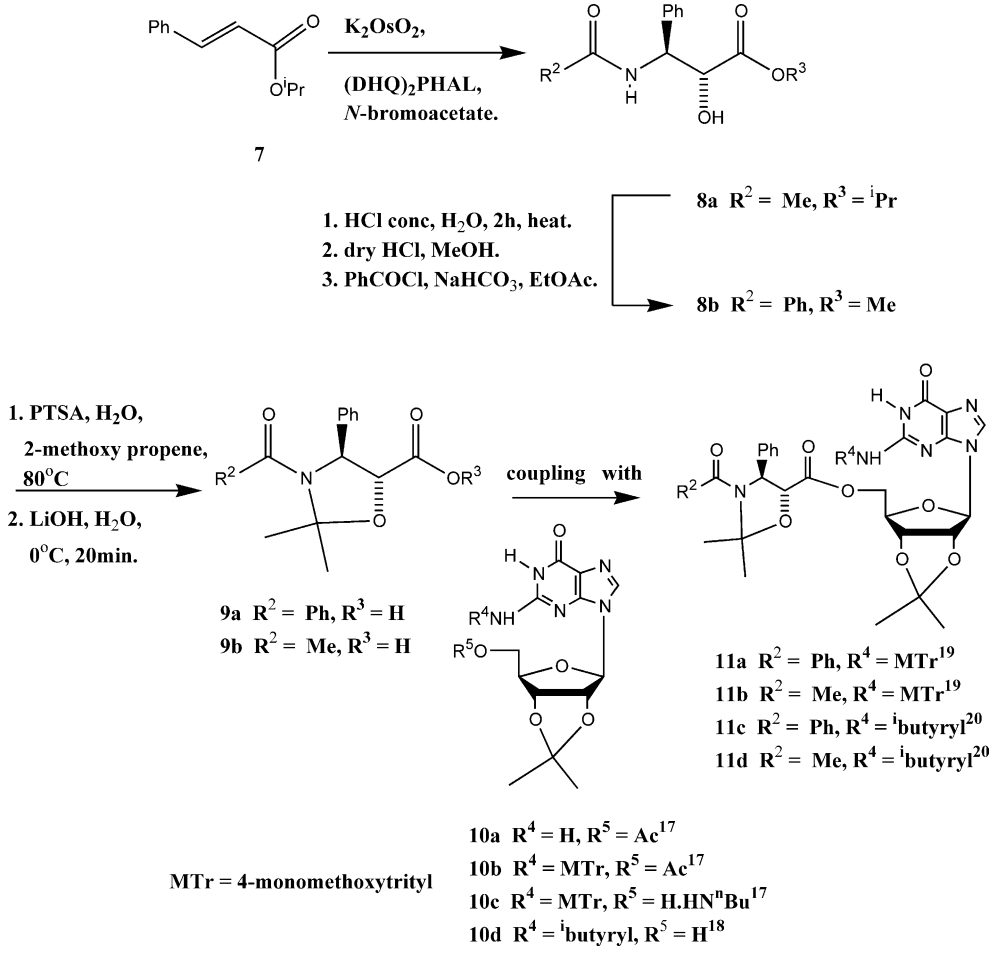
properties as taxol **1**. The advantage here, however, would be that modification of guanosine and related nucleosides has been extensively studied and hence the possibilities for modification of our hybrid molecule **6** to produce tailored anti-cancer compounds are as equally extensive. Guanosine is a cheap and readily available chemical, and the isoserine side-chain **6a** has been synthesized several times, by various routes.[14] Again, molecular modelling[10] of the hybrid molecule **6** shows that it has similar shape and electronic potential distribution as taxol **1** and GTP **4**.

Using the synthesis for the taxol isoserine side-chain put forward by Sharpless,[15] (Scheme 2), we synthesized the side-chains **9a** and **9b** in good yield. The guanosine secondary hydroxyls were protected with the isopropylidene functionality[16] and then the free NH_2 group was protected with either monomethoxytrityl via the acetate **10a**[17] or isobutyryl groups[18] to give **10c** and **10d** respectively.[19]

During our initial studies in this area, we synthesized four precursors, **11a–d**, to molecule **6**[19,20] through



Scheme 1.



Scheme 2.

coupling side-chain **9a** or **9b** to **10c** or **10d** (Scheme 2). When tested in vitro these molecules show activity (IC5Os) against the colon cancer cell line SW480. The cytotoxic assays were performed according to the microculture MTT method.[21] Two compounds, **11a** and **11b** have IC_{50}s of l52.45 and l9.87 μm, respectively. Compounds **11c** and **11d** are also active with IC_{50}s of 221.64 and 192.41 μm, respectively. The IC_{50} value for taxol when tested against SW480 is 0.312 μm.[21] Tests on the protected guanosines **10a–d**, and protected isoserine molecules **9a** and **9b**, before they are coupled to form **11a–d**, show no activity against the cell line SW480.

In conclusion, it would appear that taxol may have a biosteric and bioelectronic relationship to GTP, and that the hypothesis put forward above has credibility as shown by the bioactivity that molecules **11a–d** possess. It would not be surprizing that compounds **11a–d** are not as active as taxol against SW40, as C-2′ hydroxyl protected taxols often show significantly reduced activity compared to taxol.[5] However, the real comparison and proof of concept would need to be supported not only by cytotoxic assays but also by microtuble (dis)-assembly assays. It is quite possible that these new GTP mimics might have cytotoxic effects without any relation to taxol mode of binding. We are in the process of carrying out these microtuble (dis)assembly assays. Alternatively, we have fortuitously combined two components, a simple guanosine derivative and a protected isoserine derivative, to form a new class of molecules with cytotoxic and possible anti-cancer properties.

In either case, we have produced potentially useful molecules that are relatively easy to construct, have wide possibilities for modification, and are comparatively cheap to produce. We are currently undertaking further investigation into these compounds.

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19. **Preparation of 2-*N*-(4-methoxyphenyldiphenylmethyl)-2′,3′-*iso*propylidene-5′-*O*-((4″*S*,5″*R*)-*N*-benzoyl-2″,2″-dimethyl-4″-phenyl-1″,3″-oxazolidinyl-5″-carboxylate)-guanosine 11a**. Oxazolidine **9a** (0.30 g, 0.92 mmol) and dicyclohexylcarbodiimide (0.20 g, 0.98 mmol) were dissolved in toluene (1.5 mL) with stirring, at 20 °C, for 1 h. Butylammonium guanosine **10c** (0.37 g, 0.55 mmol) and 4-dimethylaminopyridine (0.04 g, 0.31 mmol) were dissolved in toluene (3 mL) and added to the reaction mixture. Stirring was continued for 10 min, then the reaction temperature was elevated to 80 °C. Heating was maintained for 2 h, the reaction was allowed return to ambient temperature, the saturated sodium bicarbonate solution (7 mL) was added. The aqueous layer was extracted with dichloromethane (3×5 mL) and combined organics were washed with brine, dried ($MgSO_4$), filtered and concentrated (<30 °C) to give crude **11a**. This was purified on silica gel, eluting with (1:8:12) (methanol/chloroform/hexanes) to yield **11a** (0.352 g, 71%), white powder.

$C_{52}H_{45}N_6O_9$ requires (%): C 69.57, H 5.02, N 9.36; found: C 69.32, H 5.07, N 9.22. R_f 0.20 (1:8:12 methanol/chloroform/hexanes); ν_{max} (KBr) 3314, 3061, 2932, 1748, 1693, 1645 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, 40 °C) δ 1.28 (3H, s, acetonide), 1.51 (3H, s, acetonide), 1.80 (3H, s, oxazolidine), 1.91 (3H, s, oxazolidine), 3.72 (3H, s, ArOC$\underline{H}_3$), (1H, m, H-5′), 4.25 (2H, m, H-4′, H-5‴), 4.48 (1H, bd, $J_{3'\text{-}2'}$ = 5.9 Hz, H-3′), 4.52 (1H, d, $J_{5''\text{-}4''}$ = 5.9 Hz, H-5″), 4.96 (1H, bd, $J_{2'\text{-}3'}$ = 5.9 Hz, H-2′), 5.24 (1H, d, $J_{4''\text{-}5''}$ = 5.9 Hz, H-4″), 5.61 (1H, d, $J_{1'\text{-}2'}$ = 3.9 Hz, H-1′), 6.76 (3H, d overlapping bs, J = 9.9 Hz, ArCH-

CHCOMe, NH), 6.91 (2H, m, aromatic), 7.12–7.33 (22H, m, aromatic, NH, H-8); ^{13}C NMR (100 MHz, $CDCl_3$), δ 25.58, 25.76, 26.12, 27.28, 55.10, 64.72, 65.28, 70.65, 80.94, 81.16, 82.26, 82.73, 90.51, 97.90, 113.19, 114.22, 118.36, 126.08, 126.83, 126.93, 127.80, 127.90, 128.03, 128.11, 128.49, 128.66, 128.98, 129.35, 130.10, 136.87, 137.47, 138.61, 144.44, 149.79, 151.35, 158.31, 168.84, 169.16.

Preparation of 2-*N*-(4-methoxyphenyldiphenylmethyl)-2′,3′-*iso*-propylidene-5′-*O*-((4″*S*,5″*R*)-*N*-acetyl-2″,2″-dimethyl-4″-phenyl-1″,3″-oxazolidinyl-5″-carboxylate)-guanosine 11b: As for **11a** using 9b, yield 78%, white powder.

$C_{49}H_{43}N_6O_9$ requires (%): C 68.45, H 5.00, N 9.78; found: C 68.58, H 5.11, N 9.63. R_f 0.45(1:19 methanol/chloroform); ν_{max} (KBr) 3333, 3059, 2987, 1744, 1695 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6), δ 1.20 (3H, s, acetonide), 1.38 (3H, s, acetonide), 1.56 (3H, s, CH$_3$CON), 1.66 (3H, s, oxazolidine), 1.70 (3H, s, oxazolidine), 3.69 (3H, s, ArOCH$_3$), 3.95 (1H, dd, $J_{5'\text{-}5'''}$ = 11.8 Hz, $J_{5'\text{-}4'}$ = 4.9 Hz, H-5′), 4.05 (1H, dd, $J_{5'''\text{-}5'}$ = 11.8 Hz, $J_{5'''\text{-}4'}$ = 3.9 Hz, H-5‴), (1H, m, H-4′), 4.24 (1H, dd, $J_{3'\text{-}2'}$ = 6.4 Hz, $J_{3'\text{-}4'}$ = 2.9 Hz, H-3′), 4.54 (1H, d, $J_{4''\text{-}5''}$ = 3.9 Hz, H-4″), 4.82 (1H, dd, $J_{2'\text{-}3'}$ = 6.4 Hz, $J_{2'\text{-}1'}$ = 3.5 Hz, H-2′), 5.28 (1H, d, $J_{5''\text{-}4''}$ = 3.9 Hz, H-5″), 5.49 (1H, d, $J_{1'\text{-}2'}$ = 3.5 Hz, H-1′), 6.85 (2H, d, J = 8.9 Hz, ArCHCHOMe), 7.16–7.54 (19H, m, aromatic, 2×NH), 7.70 (1H, s, H-8); ^{13}C NMR (100 MHz, $CDCl_3$), δ 24.72, 25.74, 25.80, 25.95, 27.29, 55.09, 64.14, 64.79, 70.64, 81.23, 81.66, 82.17, 82.63, 90.56, 98.40, 113.16, 114.23, 118.45, 126.05, 126.91, 127.87, 128.34, 128.65, 129.22, 130.10, 136.34, 136.87, 139.70, 144.52, 149.78, 151.39, 158.29, 168.54, 169.74.

20. **Preparation of 2-*N*-*iso*butyryl-2′,3′-*iso*propylidene-5′-*O*-((4″*S*,5″*R*)-*N*-benzoyl-2″,2″-dimethyl-4″-phenyl-1″,3″-oxazolidinyl-5″-carboxylate)-guanosine 11c**. (4*S*,5*R*)-*N*-Benzoyl-2,2-dimethyl-4-phenyl-1,3-oxazolidinyl-5-carboxylic acid **9a** (0.33 g, 1.00 mmol) and dicyclohexylcarbodiimide (0.23 g, 1.10 mmol) were stirred together at 0 °C in toluene (1.5 mL) for 30 min. A solution of **10d** (0.25 g, 0.64 mmol) and 4-dimethylaminopyridine (0.05 g, 0.38 mmol) in chloroform (4 mL) was added in one portion at 20 °C and stirring maintained for 10 min. The reaction mixture was heated to mild reflux. After 3 h, the reaction mixture was allowed cool to ambient temperature and saturated sodium bicarbonate added (5 mL). The aqueous layer was extracted with dichloromethane (2×7 mL) and the combined organic extracts washed with brine, dried ($MgSO_4$), filtered and concentrated. The crude product indicated complete conversion by tlc (1:9 methanol/chloroform) and was purified by flash column chromatography (eluant 4% methanol in chloroform) to yield **11c** as a white powder (0.40 g, 90%).

$C_{36}H_{35}N_6O_9$ requires: C 62.16, H 5.04, N 12.09; found: C 62.10, H 5.08, N 12.21. R_f 0.33 (1:24 methanol/chloroform); ν_{max} (KBr) 3380, 3112, 2961, 1750, 1687 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$), δ 1.25 (6H, m, (CH$_3$)$_2$CH), 1.39 (3H, s, acetonide), 1.61 (3H, s, acetonide), 1.87 (3H, bs, oxazolidine), 1.97 (3H, s, oxazolidine), 2.69 (1H, septet, J = 6.9 Hz, (CH$_3$)$_2$CH), 4.08 (1H, dd, $J_{5'\text{-}5'''}$ = 11.4 Hz, $J_{5'\text{-}4'}$ = 5.9 Hz, H-5′), 4.42 (1H, m, H-4′), 4.63 (1H, d, $J_{5''\text{-}4''}$ = 6.4 Hz, H-5″), 5.08 (2H, m, H-3′, H-5‴), 5.13 (1H, dd, $J_{2'\text{-}3'}$ = 6.4 Hz, $J_{2'\text{-}1'}$ = 1.5 Hz, H-2′), 5.22 (1H, bs, H-4″), 5.99 (1H, d, $J_{1'\text{-}2'}$ = 1.5 Hz, H-1′), 6.95 (2H, m, aromatic), 7.11–7.33 (8H, m, aromatic), 7.73 (1H, s, H-8), 9.40 (1H, s, NH), 12.11 (1H, s, NH); ^{13}C NMR (100 MHz, $CDCl_3$), δ 18.81, 19.01, 24.92, 25.42, 25.56, 27.14, 36.33, 64.22, 65.41, 80.96, 81.76, 84.93, 85.05, 90.56, 97.96, 114.57, 122.13, 126.03, 126.77, 127.91, 128.00, 128.52, 129.41, 137.45, 137.95, 138.68, 147.25, 147.90, 155.36, 169.06, 170.22, 179.03.

Preparation of 2-*N*-*iso*butyryl-2′,3′-*iso*propylidene-5′-*O*-((4″*S*,5″*R*)-*N*-acetyl-2″,2″-dimethyl-4″-phenyl-1″,3″-oxazolidinyl-5″-carboxylate)-guanosine 11d. As for **11c** using **9b**, yield 39%, white powder.

$C_{33}H_{33}N_6O_9$ requires (%): C 60.27, H 5.02, N 12.78; found: C 60.12, H 5.11, N 12.60. R_f 0.08 (1:9:15 methanol/chloroform/hexanes); ν_{max} (KBr) 3423, 3168, 2982, 2936, 1744, 1686, 1610, 1560 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$), δ 1.25 (6H, m, (CH$_3$)$_2$CH), 1.35 (3H, s, acetonide), 1.61 (3H, s, acetonide), 1.77 (3H,s, CH$_3$CO), 1.82 (3H, s, oxazolidine), 1.86 (3H, s, oxazolidine), 2.71 (1H, septet, J = 6.9 Hz, (CH$_3$)$_2$CH), 4.13 (1H, dd, $J_{5'\text{-}5'''}$ = 11.3 Hz, $J_{5'\text{-}4'}$ = 4.9 Hz, H-5′), 4.50 (1H, m, H-4″), 4.61 (1H, d, $J_{5''\text{-}4''}$ = 3.9 Hz, H-5″), 5.13 (3H, m, H-2′, H-3′. H-5‴), 5.33 (1H. d, $J_{4''\text{-}5''}$ = 3.9 Hz, H-4″), 6.03 (1H, d, $J_{1'\text{-}2'}$ = 1.0 Hz, H-1′), 7.25–7.35 (5H, m, aromatic), 7.74 (1H, s, H-8), 9.46 (1H, s, NH), 12.13 (1H, s, NH); ^{13}C NMR (100 MHz, $CDCl_3$), δ 18.81, 19.07, 24.60, 25.36, 26.16, 26.32, 27.18, 36.30, 64.19, 64.53, 81.79, 81.85, 85.14, 90.45, 98.79, 114.64, 122.21, 125.88, 128.47, 129.28, 138.01, 139.77, 147.20, 147.96, 155.33, 168.31, 171.45, 179.11.

21. Quintero, A.; Pelcastre, A.; Solano, J. D. *J. Pharm. Pharmaceut. Sci.* **1999**, *2*, 108.