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^{1}H and ^{13}C assignments of 6-, 8-, 9- substituted purines

1 | INTRODUCTION

Purines are heterocyclic aromatic organic compounds consisting of two 5- and 6-membered fused rings containing nitrogen. They are present in numerous compounds, including natural products, with potent biological activity. Purine analogs are used for the treatment of acute leukemias. Thiopurine derivatives are effective antiviral (acyclovir and ganciclovir) or antitumor agents such as vidarabine, among other clinical uses.[1]

Over the years, the purine nucleus has become one important pharmacophoric group. It is capable of interfering in the synthesis and function of enzymes and nucleic acids. It is also frequently used in the development of protein kinase inhibitors.[2]

Our research group has developed new routes to produce purine libraries. One of such routes is the one-pot synthesis[3] to obtain 6-, 8-, and 9-substituted purines from 4-alkylamino-5-amino-6-chloropirimidines, alcohols, and N,N-dimethylamides. We also presented a new approach,[4] to obtain trisubstituted purines. We were able to prepare a library of polysubstituted purines. Some compounds from this library were found to be specific inhibitors of the death-associated protein kinase-1.[5] The leading compounds of this library are potent inducers of apoptosis in tumor lymphocytes and also reduce viability of trypanosomes.[4,6]

We herein report the unambiguous assignment of ^{1}H and ^{13}C of a subset of these new purine derivatives. Assignments were carried out in compounds **3 k** and **3n** which have, as substituents, phenyl and benzyl at Positions 6 and 8, respectively. We also characterize compound **3o**, which has benzyl, phenyl, and phenethyl substituents at positions 6, 8, and 9, respectively. We therefore provide better knowledge of the molecular structure of our compounds, while obtaining further insight for future structure–activity studies and for improving the structural determination of other purine derivatives.

2 | EXPERIMENTAL

2.1 | Synthesis

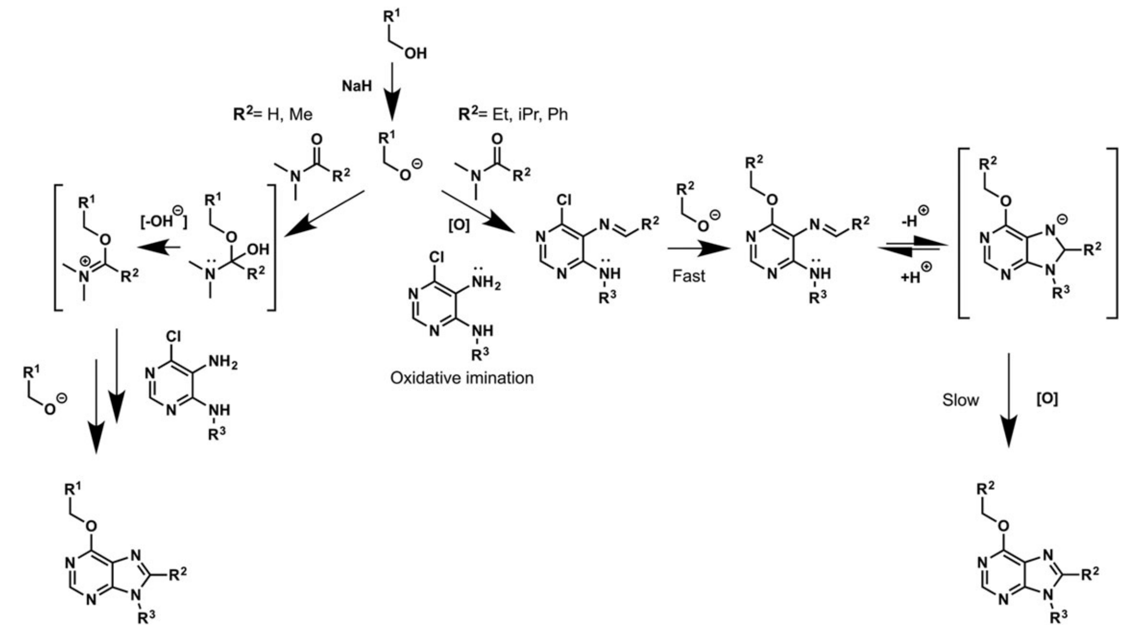
Purines were prepared as described elsewhere [4,5]

Scheme 1 shows the previously reported synthetic pathway to obtain the novel family of purine derivatives **3a-o**. Trisubstituted purines at Positions 6, 8, and 9 were prepared starting from 6-chloro-4,5-diaminopyrimidine, alcohols, and *N*, *N*-dimethylamides in basic conditions without metal catalysis. Depending on the size of the R^1 substituent of the amides (Scheme 1), two different synthetic routes may occur. Route A results in purine analogs whose C8 substituents are derived from the amide, whereas Route B gives rise to purines with C8 substituents coming from the alcohol. Route A proceeds via in situ generation of N-alkylimidate species, which are created by reaction between amides and alkoxides leading to purine derivatives with either H or methyl in C8 when using *N*,*N*-dimethylformamide or *N*,*N*-dimethylacetamide, respectively. When steric hindrance of amides increases, such as in the cases of *N*,*N*-dimethylbenzamide or *N*,*N*-dimethylpropionamide, Route A is then impeded and a metal-free tandem alcohol oxidation/annulation reaction occurs giving rise to Route B products. Therefore, competition between N-alkylimidate formation and metal-free oxidative coupling of primary alkoxides and diaminopyrimidines with Schiff base formation and subsequent annulation can be controlled. In both routes S_NAr of the chloro atom at C6 by alkoxides takes place and along with the R^3 group of exocyclic nitrogen located at C4 of the starting pyrimidine, increases structural diversity of these one-pot synthesis.

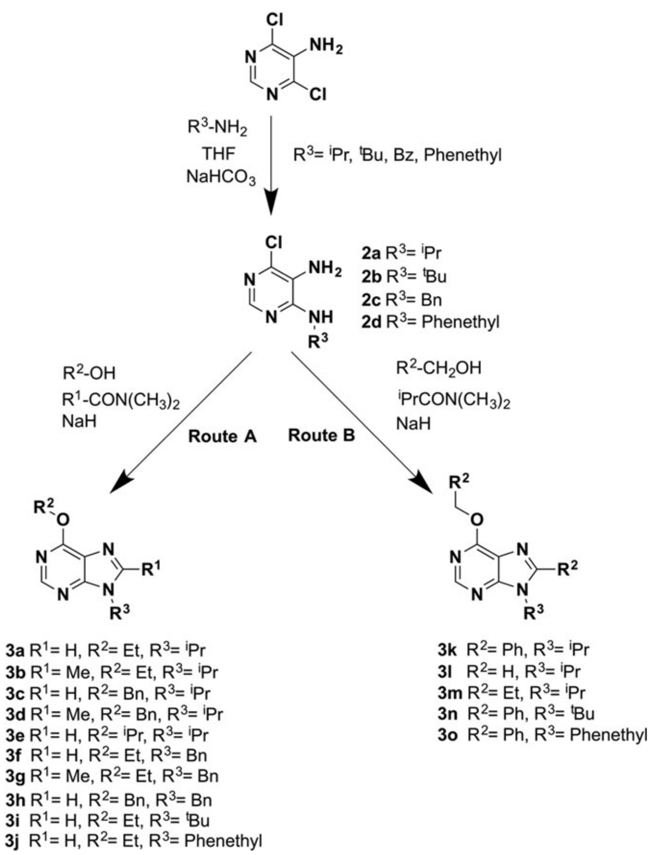
This synthetic platform, therefore, allows the creation of a diversity of purine analogues in a parallel and straightforward fashion using a variety of amides and alcohols with different pyrimidines under the same reaction conditions. Scheme 2 shows the set of 15 purines presented here, which were obtained through either Route A or B.

2.2 | Nuclear magnetic resonance techniques

^{1}H and ^{13}C NMR data (chemical shifts multiplicity and coupling constants) for compounds **3a-n** are shown in Tables below. Unambiguous assignments for all NMR signals were accomplished by combined analysis of ^{1}H, ^{13}C,



SCHEME 1 Proposed mechanisms of action for Routes A and B



SCHEME 2 Synthetic routes to generate purine types "A" or "B" and set of 15 purines prepared through either route a or B

90° distortionless enhancement by polarization transfer (DEPT), heteronuclear single quantum coherence (HSQC) and heteronuclear multiple bond coherence (HMBC), correlation spectroscopy (COSY), and total correlation spectroscopy (TOCSY) NMR experiments.

^{1}H Nuclear magnetic resonance spectra were recorded on a Varian Inova Unity (300 MHz), Varian Direct Drive (400 MHz), and/or Varian Direct Drive (500 MHz). Chemical shifts (δ) are referenced to the residual solvent peak: $CDCl_3$, δ 7.26 (^{1}H), δ 77.16 (^{13}C). Spin multiplicities are given as s (singlet), bs (broad singlet), d (doublet), dd (double doublet), ddd (double double doublet), t (triplet), dt (double triplet), pt (pseudotriplet), q (quadruplet), and m (multiplet). Coupling constants (*J*) are given in Hz. ^{13}C NMR were recorded on a Varian Direct Drive (400 MHz) and Varian Direct Drive (500 MHz). DEPT experiments were carried out using the standard pulse sequence.[7] The HMBC, HSQC, H2BC, COSY, TOCSY, and DEPT spectra were measured with a pulse sequence gc2hmbc, gc2hsqcse, gc2h2bc, gCOSY, gTOCSY, and gDEPT, respectively (Standard sequence Agilent Vnmrj 4.2A software), CRISIS type.[8]

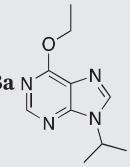
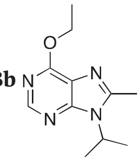
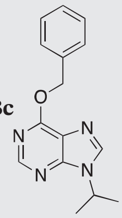
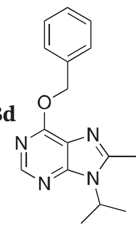
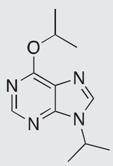
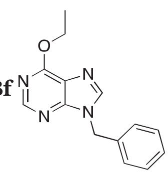
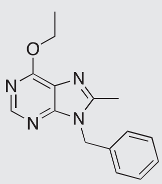
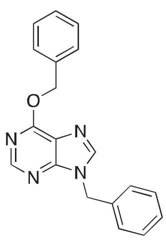
3 | RESULTS AND DISCUSSION

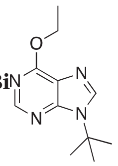
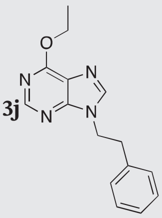
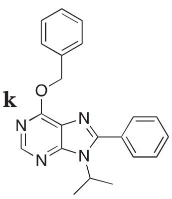
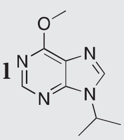
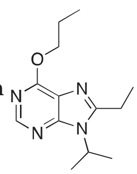
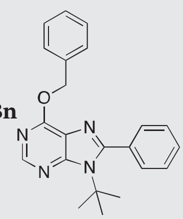
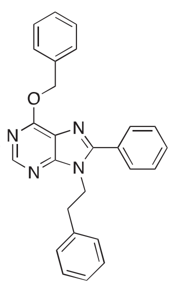
3.1 | Analysis of ^{1}H and ^{13}C-NMR spectra

To facilitate the analysis of all purines, NMR data are presented in tables. Substituent at 6, 8, and 9 positions of the purine ring are named as R^1, R^2, and R^3, respectively. Table 1 and 2 show ^{1}H and ^{13}C-NMR chemical shifts (δ) for compounds **3a-o**.

Table 3 shows the family of compounds derived from purine as well as the number scheme of the compounds used.

TABLE 1 [1]H-NMR chemical shifts (δ) and coupling constants (J, Hz) of purine derivatives (**3a-o**)

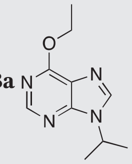
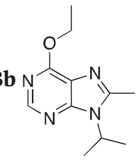
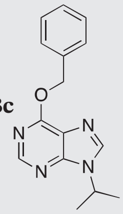
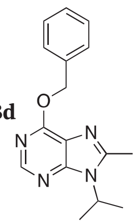
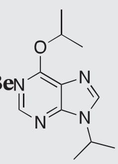
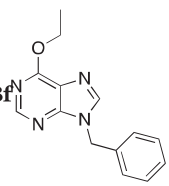
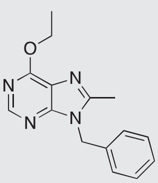
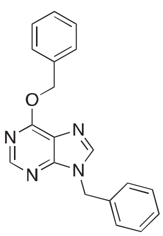
Compound	H-2	H-8	R_1	R_2	R_3
3a 	8.48 (s)	7.96 (s)	4.63 (q, 7), 1.47 (t, 7)	—	4.85 (m), 1.59 (d, 7)
3b 	8.42 (s)	—	4.62 (q, 7), 1.49 (t, 7)	2.64 (s)	4.74 (m), 1.68 (d, 6.5)
3c 	8.54 (s)	7.99 (s)	5.68 (s), 7.54 (H2′-6′, d, 7.5), 7.36 (H3′-5′, dd, 7.5, 1.5), 7.31 (H4′, dd, 7.5, 1.5)	—	4.89 (m), 1.63 (d, 7)
3d 	8.46 (s)	—	5.64 (s), 7.53 (H2′-6′, d, 7.5), 7.34 (H3′-5′, dd, 7.5, 2), 7.30 (H4′, dd, 7.5, 2)	2.64 (s)	4.74 (m), 1.68 (d, 7)
3e 	8.51 (s)	7.95 (s)	5.67 (m), 1.62 (d, 6.90)	—	4.88 (m), 1.47 (d, 6)
3f 	8.55 (s)	7.88(s)	4.66 (q, 7), 1.51 (t, 7)	—	5.40 (s), 7.35–7.30 (m), 7.27 (dd, 8)
3 g 	8.50 (s)	—	4.65 (q, 7), 1.51 (t, 7)	2.51 (s)	5.40 (s), 7.32 (H4′, dd, 6, 2), 7.28 (H3′-5′, dd, 6, 2), 7.14 (H2′-6′, dd, 6, 2)
3 h 	8.58 (s)	7.89 (s)	5.69 (s), 7.54 (d, 7.5), 7.38–7.34 (m)	—	5.41 (s), 7.34–7.30 (m), 7.26 (dd, 8)

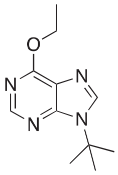
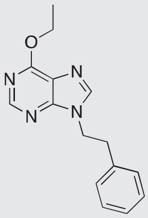
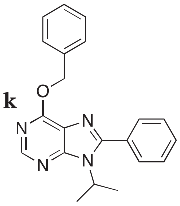
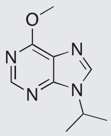
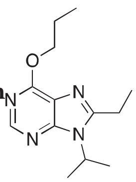
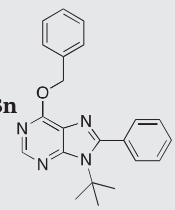
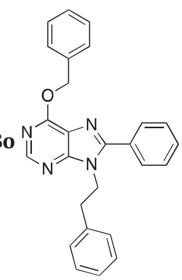
Compound	H-2	H-8	R_1	R_2	R_3
3i 	8.50 (s)	7.98 (s)	4.65 (q, 7.2), 1.51 (t, 7.2)	—	1.81 (s)
3j 	8.52 (s)	7.50 (s)	4.64 (q, 7.21), 1.49 (t, 7.21)	—	7.25–7.17 (m), 7.02 (dd, 8.41), 4.46 (t, 7.21), 3.16 (t, 7.21)
3 k 	8.54 (s)	—	5.69 (s), 7.66 (m), 7.37–7.29 (m)	7.57–7.50 (m)	4.76 (m), 1.73 (d, 6.90)
3 l 	8.53 (s)	7.98 (s)	4.18 (s)	—	4.89 (m), 1.62 (d, 6.5)
3 m 	8.43 (s)	—	4.52 (t, 7.20), 1.26 (m), 1.05 (t, 7.50)	2.94 (q,7.2), 1.43 (t, 7.2)	4.69 (m), 1.70 (d, 6.90)
3n 	8.55 (s)	—	5.65 (s), 7.54 (d, 7.5), 7.35–7.27 (m)	7.46 (d, 7.5), 7.45–7.38 (m)	1.66 (s)
3o 	8.58 (s)	—	5.71 (s), 7.55 (d, 5.2), 7.37–7.31 (m)	7.48 (d, 4.4), 7.45–7.43 (m)	7.25–7.17 (m), 6.91 (dd, 2.4), 4.56 (t, 4.8), 3.10 (t, 4.8)

Note: NMR = nuclear magnetic resonance.

In particular, we focused the analysis in protons belonging to phenyl, benzyl, and phenethyl aromatic rings within a same molecule, as for compounds **3o**, **3 k**, and **3n**. TOCSY method was used to identify unequivocally these aromatic protons of our compounds. Purine **3o** was firstly analyzed. Irradiation frequency of δ 7.55 presents an associated spin system that corresponds with aromatic protons of the R^1 benzyl

TABLE 2 ^{13}C-NMR chemical shifts (δ) of purine derivatives (**3a-o**)

Compound	C_2	C_4	C_5	C_6	C_8	R_1	R_2	R_3
3a 	151.8	152.0	122.0	160.9	139.7	14.6 (CH_3), 63.1 (CH_2)	—	47.5 (CH), 22.7 (CH_3)
3b 	150.7	150.6	120.9	159.9	133.2	14.7 (CH_3), 62.8 (CH_2)	15.4	48.5 (CH), 21.4 (CH_3)
3c 	151.8	152.1	122.0	160.7	139.9	CH_2 (68.5), C-1′$_{ph}$ (136.4), C-2′,6′$_{ph}$ (128.6), C-3′,5′$_{ph}$ (128.5), C-4′$_{ph}$(128.2)	—	47,6 (CH), 22,8 (CH_3)
3d 	150.5	150.9	120.9	159.6	140.0	CH_2 (68.23), C-1′$_{ph}$ (136.59), C-2′,6′$_{ph}$ (128.60), C-3′,5′$_{ph}$ (128.52), C-4′$_{ph}$(128.15)	15.4	48.5 (CH), 21.4 (CH_3)
3e 	152.0	150.2	122.2	160.8	139.5	70.3 (CH), 22.8 (CH_3)	—	47.5 (CH), 22.2 (CH_3)
3f 	152.5	152.3	121.5	161.1	142.0	14.7 (CH_3), 63.3 (CH_2)	—	CH_2 (47.6), C-1′$_{ph}$ (135.5), C-2′,6′$_{ph}$ (129.2), C-3′,5′$_{ph}$ (127.9), C-4′$_{ph}$(128.6)
3 g 	151.7	151.6	120.5	160.0	153.7	14.5 (CH_3), 63.1 (CH_2)	14.7	CH_2 (46.2), C-1′$_{ph}$ (135.7), C-2′,6′$_{ph}$ (129.1), C-3′,5′$_{ph}$ (127.1), C-4′$_{ph}$(128.3)
3 h 	152.4	152.5	121.6	160.8	142.2	CH_2 (68.6), C-1′$_{ph}$ (136.3), C-2′,6′$_{ph}$ (128.6), C-3′,5′$_{ph}$ (128.5), C-4′$_{ph}$(128.3)	—	CH_2 (47.6), C-1′$_{ph}$ (135.4), C-2′,6′$_{ph}$ (129.3), C-3′,5′$_{ph}$ (127.9), C-4′$_{ph}$(128.7)

Compound	C_2	C_4	C_5	C_6	C_8	R_1	R_2	R_3
3i 	151.1	152.4	123.1	161.1	139.8	14.7 (CH_3), 63.0 (CH_2)	—	57.7 ($C_{4°}$), 29.2 (CH_3)
3j 	152.2	152.0	121.6	160.9	142.1	14.6 (CH_3), 63.2 (CH_2)	—	N-CH_2(45.7) CH_2(36.3), C-1′$_{ph}$ (137.3), C-2′,6′$_{ph}$ (128.9), C-3′,5′$_{ph}$ (128.8), C-4′$_{ph}$ (127.2)
3 k 	150.9	153.7	122.0	160.4	153.0	CH_2 (68.3), C-1′$_{ph}$ (136.6), C-2′,6′$_{ph}$ (128.7), C-3′,5′$_{ph}$ (128.5), C-4′$_{ph}$(128.2)	C-1′$_{ph}$ (135.5), C-2′,6′$_{ph}$ (129.7), C-3′,5′$_{ph}$ (128.9), C-4′$_{ph}$(130.3)	49.9 (CH), 21.4 (CH_3)
3 l 	151.9	148.8	122.0	161.2	139.9	54.3	—	47.6 (CH), 22.8 (CH_3)
3 m 	150.6	150.2	133.9	160.2	155.2	OCH_2(68.6) CH_2 (22.4) CH_3(10.5)	12.4 (CH_3), 22.1 (CH_2)	48.5 (CH), 21.4 (CH_3)
3n 	150.3	154.4	121.7	160.5	153.3	CH_2 (68.3), C-1′$_{ph}$ (136.5), C-2′,6′$_{ph}$ (128.8), C-3′,5′$_{ph}$ (128.5), C-4′$_{ph}$(128.18)	C-1′$_{ph}$ (134.9), C-2′,6′$_{ph}$ (130.0), C-3′,5′$_{ph}$ (128.0), C-4′$_{ph}$(129.6)	60.9 ($C_{4°}$), 31.0 (CH_3)
3o 	151.7	154.0	121.4	160.3	153.3	CH_2 (68.5), C-1′$_{ph}$ (136.4), C-2′,6′$_{ph}$ (128.6), C-3′,5′$_{ph}$ (128.5), C-4′$_{ph}$(128.2)	C-1′$_{ph}$ (129.7), C-2′,6′$_{ph}$ (129.3), C-3′,5′$_{ph}$ (129.8), C-4′$_{ph}$(130.3)	N-CH_2(45.6) CH_2(35.7), C-1′$_{ph}$ (137.2), C-2′,6′$_{ph}$ (128.8), C-3′,5′$_{ph}$ (128.8), C-4′$_{ph}$ (127.0)

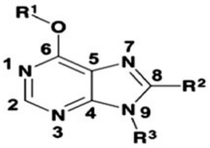
Note: NMR = nuclear magnetic resonance.

substituent located at C6 of the purine ring. In the case of irradiation frequency of δ 7.50, the associated spin system corresponds to protons of the aromatic ring appearing at Position 8 of the purine ring (R^2), a phenyl substituent. Finally, irradiation frequency of δ 7.19 present an associated spin system that corresponds to protons of the R^3 substituent located at C9 of the purine ring, a phenethyl group.

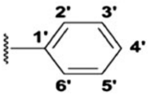
Once chemical shifts of the protons of the three types of aromatic rings at purine substituents were identified,

TABLE 3 Summary of ^{13}C-NMR chemical shifts (δ) of purine derivatives (**3a-o**)

Compound	C_2	C_4	C_5	C_6	C_8
3i-o	151.9–152.2	148.8–154.4	121.4–133.9	160.2–161.2	139.8–155.2



3a: R^1= Et, R^2= H, R^3= iPr
3b: R^1= Et, R^2= Me, R^3= iPr
3c: R^1= Bn, R^2= H, R^3= iPr
3d: R^1= Bn, R^2= Me, R^3= iPr
3e: R^1= iPr, R^2= H, R^3= iPr
3f: R^1= Et, R^2= H, R^3= Bn
3g: R^1= Et, R^2= Me, R^3= Bn
3h: R^1= Bn, R^2= H, R^3= Bn
3i: R^1= Et, R^2= H, R^3= tBu
3j: R^1= Et, R^2= H, R^3= Phenethyl
3k: R^1= Bn, R^2= Ph, R^3= iPr
3l: R^1= Me, R^2= H, R^3= iPr
3m: R^1= Pr, R^2= Et, R^3= iPr
3n: R^1= Bn, R^2= Ph, R^3= tBu
3o: R^1= Bn, R^2= Ph, R^3= Phenethyl



Ph, Bn or Phenethyl

Note: NMR = nuclear magnetic resonance.

coupling techniques to identify C–H and H–H interactions (HSQC, HMBC, COSY, TOCSY, and H2BC) were used to unequivocally assign protons of the three different aromatic rings at 6, 8, and 9 positions of compound **3o** as shown in Table 1. Therefore, by means of TOCSY studies, we unequivocally assigned the protons of the aromatic rings, which appear in Positions 6, 8, and 9 of the purine ring (Table 1). A similar procedure was carried out to assign the aromatic protons of compounds **3 k** and **3n.**

Table 2 and 3 shows the ^{13}C-NMR data. Similar to our ^{1}H-NMR analysis, unambiguous assignment of ^{13}C chemical shifts was performed for the three aromatic rings, phenyl, benzyl, and phenethyl substituents at positions 6, 8, and 9 of purine **3o**, as well as for carbons of the aromatic substituents, benzyl, and phenyl, located at C6 and C8 of purines **3 k** and **3n**.

For compound **3o**, quaternary carbons were assigned using 90° DEPT and ^{13}C-NMR spectra. Chemical shifts at δ 160.32, 153.96, 153.34, 137.27, 136.40, 129.66, and 121.40 correspond to C4, C5, C6, and C8 of the purine ring of phenyl, benzyl, and phenethyl rings, located at C6, C8, and N9. These assignments were confirmed by analysis of HSQC spectra. HMBC spectrum indicated that the methylene protons of the benzyl substituent located at C6 of the purine ring showed coupling with the quaternary carbon appearing at δ 160.32, corresponding to C6. The same methylene protons were coupled to the quaternary carbon of the aromatic ring substituent at C6. Carbon C1' was at δ 136.40. The proton at C2 of the purine ring (δ 8.58) is coupled long distance with C6 and with C4 at δ 153.96. The methylene protons of the phenethyl group (δ 5.71), substituent at C9 are coupled to C1' (δ 137.27) and with C4. The quaternary carbon at δ 121.40 was assigned to C5, since was not coupled with any hydrogen. The quaternary carbon δ 153.34 was assigned to C8, since in the HMBC spectrum it showed coupling to the aromatic protons of the substituent phenyl ring. The signal of the quaternary carbon at δ 129.66 was assigned to C1 of the phenyl group of the substituent at C8 of the purine ring.

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ORCID

María José Pineda de las Infantas Villatoro http://orcid.org/0000-0002-0617-6408

Álvaro Lorente-Macías[1,2]
Manuel Benítez-Quesada[1]
Ignacio J. Molina[2,3]
Asier Unciti-Broceta[4]
Juan José Díaz-Mochón[1,5]
María José Pineda de las Infantas Villatoro[1]

[1]*Departamento de Química Farmacéutica y Orgánica, Facultad de Farmacia, Campus de Cartuja, Universidad de Granada, Granada, Spain*

[2]*Instituto de Biopatología y Medicina Regenerativa, Centro de Investigación Biomedica, Parque Tecnológico de Ciencias de la Salud, Universidad de Granada, 18016 Granada, Spain*

[3]*Instituto de Investigación Biosanitaria ibs.GRANADA, Granada University Hospitals and University of Granada, Spain*

[4]Ediburgh Cancer Research UK Centre MBC Institute of Genetics and Molecular Medicine, University of Edinburgh, Crewe Road South, Edinburgh EH4, 2XR, UK

[5]Avenida de la Ilustración 114, Parque Tecnológico de Ciencias de la Salud, Centro Pfizer-Universidad de Granada-Junta de Andalucía de Genómica e Investigación Oncológica (GENYO), 18016 Granada, Spain

Correspondence

Juan José Díaz-Mochón, Departamento de Química Farmacéutica y Orgánica, Facultad de Farmacia, Campus de Cartuja, Universidad de Granada, Granada, Spain.
Email: juandiaz@ugr.es

María José Pineda de las Infantas Villatoro, Departamento de Química Farmacéutica y Orgánica, Facultad de Farmacia, Campus de Cartuja, Universidad de Granada, Granada, Spain.
Email: mjpineda@ugr.es

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