



HAL
open science

Arylbiamidines: synthesis and structural studies en route to anticancer applications

Oleksandr Grytsai, Leticia Christina Pires Gonçalves, Rostyslav Bardovskyi, Nedra Hamouda-Tekaya, Stéphane Rocchi, Cyril Ronco, Rachid Benhida

► **To cite this version:**

Oleksandr Grytsai, Leticia Christina Pires Gonçalves, Rostyslav Bardovskyi, Nedra Hamouda-Tekaya, Stéphane Rocchi, et al.. Arylbiamidines: synthesis and structural studies en route to anticancer applications. *New Journal of Chemistry*, 2021, 45 (27), pp.11893-11897. 10.1039/D1NJ01943K . hal-03870875

HAL Id: hal-03870875

<https://hal.science/hal-03870875v1>

Submitted on 11 Dec 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

Arylbiamidines: synthesis and structural studies en route to anticancer applications†

Oleksandr Grytsai,^{‡a} Leticia Christina Pires Gonçalves,^{ib} ^{‡a} Rostyslav Bardovskyi,^{‡a} Nedra Hamouda-Tekaya,^b Stéphane Rocchi,^{ib} ^b Cyril Ronco^{ib} ^{*a} and Rachid Benhida^{*ac}

Biamidines are a unique and poorly studied class of nitrogenous compounds prone to tautomerization and H-bonding. Four series of heteroaryl diarylbiamidines were synthesized and the antimelanoma activity and physicochemical properties of the resulting 37 new compounds were evaluated. The dimethylthiazolyl 3-bromophenyl biamidine derivative B6 inhibits the growth of six different melanoma cell lines, having higher activity than the positive control drug, the B-RAF inhibitor PLX4032. This study introduces diarylbiamidines as promising frameworks for drug discovery.

Biamidine (*N'*-carbamimidoylimidamide) is a functional group in which two amidine moieties are linked by a C–N bond (Fig. 1). Examination of the structural features of biamidines suggests that these compounds share the strong base character, water solubility, tautomerism capability, and coordination properties of guanidines and biguanides.^{1–3}

Guanidine is one of the most versatile functional groups in chemistry. Compounds containing this function are ubiquitous in bioactive molecules with diverse biological properties.^{4,5} Similarly, biguanides are known for their broad biological activity and diverse therapeutic applications, e.g. antidiabetic, antimalaria, antiseptic and anticancer activity.^{6,7} Although the features of guanidine and biguanides, which contain three and five nitrogen atoms, are well known, knowledge on the four nitrogen-containing biamidines is lacking. Notably, details on the synthesis and properties of biamidines are scarce in the literature.^{8–11} Here, we report a new class of compounds, the diarylbiamidines, a general and efficient synthetic protocol

leading to different derivatives and we present their *in vitro* antimelanoma activity. Besides, we demonstrate their peculiar tautomerism and H-bonding features, relevant for drug discovery.

According to the Lipinski's rule of five, increasing the lipophilicity of biamidines may enhance their bioactivity. Hence, we designed derivatives in which a thiazolyl substituent is linked to the N-atom of the biamidine group and a second aryl group is attached to the C-atom (Fig. 2a). This functionalization is expected to increase the lipophilicity of the resulting biamidine and to affect its basicity and H-bonding capability. Thiazolyl group is found in a variety of drugs, such as antitumor, antifungal, and anti-inflammatory therapeutics,^{12–15} and its electronic and steric properties can be tuned by adding substituents at positions 4 and 5 of the heterocyclic ring.¹⁴ The aromatic substituent R² enables the fine tuning of the physicochemical properties of the derivatives, and their effect on the biological activity.

No general approach is available towards the synthesis of arylbiamidines, which were mainly obtained as side-products.^{8–10} Therefore, a new synthetic method was developed to obtain the diarylbiamidines. For that, the guanidine intermediates **6–9** were first prepared in >70% yields by thiazole-Hantzsch synthesis from amidinothiourea **5** and α -haloketones **1–4** (Fig. 2a, see ESI†). Next, these 2-thiazolylguanidines were coupled to aryl nitriles in DMF using sodium hydride as base. Full conversion was obtained in 2–6 h at 25 °C, resulting in the desired arylbiamidines within

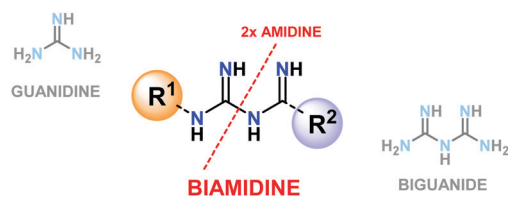


Fig. 1 Comparison of the chemical structures of the biamidine (4× N) function to the amidine (2× N), guanidine (3× N), and biguanide (5× N) functions.

^a Institut de Chimie de Nice CRNS UMR7272, Université Côte d'Azur, Nice, France. E-mail: cyril.ronco@univ-cotedazur.fr, rachid.benhida@univ-cotedazur.fr

^b Centre Méditerranéen de Médecine Moléculaire (C3M), INSERM, U1065, Université Côte d'Azur, Nice, France

^c Department of Chemical and Biochemical Sciences, Green Process Engineering, CBS, Mohamed VI Polytechnic University, UM6P, 43150 Ben Guerir, Morocco

† Electronic supplementary information (ESI) available. CCDC 2042663. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/d1nj01943k

‡ These authors contributed equally.

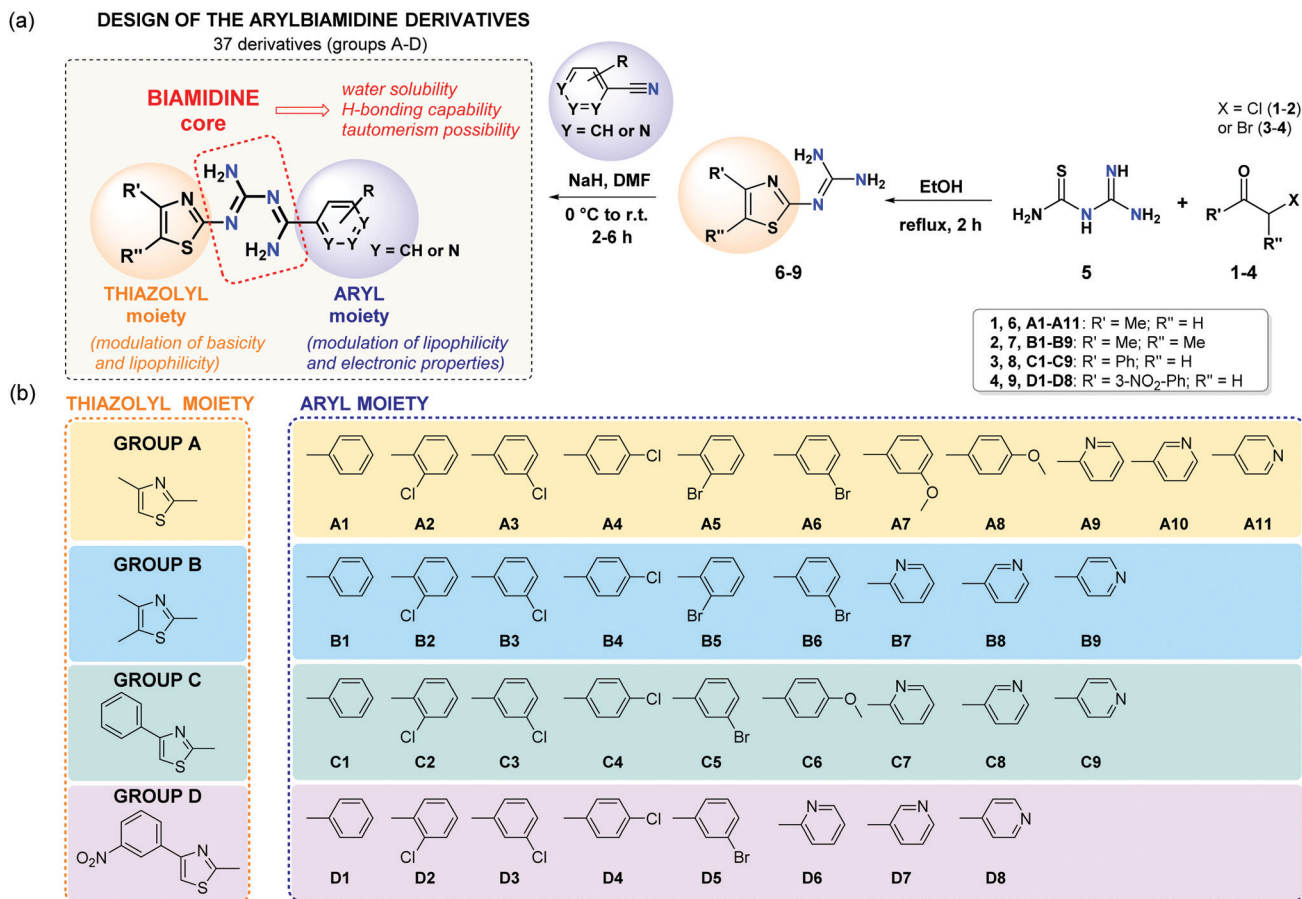


Fig. 2 Arylbiamidines: (a) Design and synthetic route for the obtention of arylbiamidines containing the biamidine core and two aryl groups. (b) Scope of arylbiamidines synthesized in this work.

up to 95% isolated yield. The use of other bases, Brønsted and Lewis acids, heating, and other solvents was investigated but did not result in the desired product (see ESI[†]). By using this approach, we synthesized 37 novel arylbiamidines within four different thiazole-substituted groups, namely groups A (4-Me-thiazolyl), B (4,5-diMe-thiazolyl), C (4-Ph-thiazolyl) and D (4-(*m*-NO₂)Ph-thiazolyl) (Fig. 2b). All compounds are air-stable as solids and in solution (DMSO, THF, DCM, PB pH 7, DMEN-PS + FBS cell media, see ESI[†]).

Experimental evidence on the solubility of the arylbiamidines in apolar solvents such as dichloromethane and diethyl ether indicated higher lipophilicity than expected. To rationalize these observations and to evaluate the effect of the addition of the thiazolyl group on the physicochemical properties of biamidines, we performed predictions of pK_{aH} , $\log D$ and $\log S$ of different biamidines, in comparison to guanidines and biguanides (Table 1, see ESI[†]). Biguanides are known to be slightly less basic than guanidines (less than 1 log unit); however, biamidines are substantially weaker bases with a difference of $\approx 2pK_{aH}$ log units. Substitution with a phenyl leads to a decrease in basicity in all classes of compounds, with phenylbiamidine showing lower basicity and higher polarity than phenylguanidine and *o*-tolylbiguanide. Similarly, the introduction of the thiazolyl group leads to a significant reduction in basicity (Table 1). As expected,

Table 1 Determination of pK_{aH} , $\log D$ and $\log S$ of biamidines in comparison to guanidines and biguanides

| Compound | ${}^s_pK_{aH}$ (exp.) ^a | pK_{aH} (calc.) ^b | $\log D$ (pH 7) ^b | $\log S$ (pH 7) ^b |
|--|------------------------------------|--------------------------------|------------------------------|------------------------------|
| Guanidine | 13.09 ± 0.11 | 11.40 | -3.65 | 2.42 |
| Phenyl | n.d. | 9.70 | -1.45 | 0.37 |
| diMe-thiazolyl 7 | 7.30 ± 0.02 | 7.30 | -0.7 | 0.25 |
| Biamidine | n.d. | 9.00 | -5.57 | 1.63 |
| R ¹ -Phenyl | n.d. | 8.30 | -3.22 | 0.00 |
| R ¹ ,R ² -Diphenyl | n.d. | 6.85 | 0.45 | -1.32 |
| diMe-thiazolyl B1 | 7.00 ± 0.02 | 6.40 | 0.73 | -1.92 |
| Biguanide | n.d. | 11.00 | -4.17 | 2.15 |
| <i>o</i> -Tolyl | 10.94 ± 0.02 | 10.20 | -1.58 | 0.00 |
| Diphenyl | n.d. | 6.77 | 0.49 | -1.54 |

^a pK_{aH} experimental (exp.) values were determined with two different potentiometric procedures (see ESI); pK_{aH} (couples amidine/amidine-H⁺); the ${}^s_pK_{aH}$ value in the pH scale is relative to each acetonitrile-water mixture. ^b Calculated values were predicted with the ChemAxon online calculator (see ESI). $\log D$ values refer to $\log P$ estimations at pH 7 and $\log S = \log$ (solubility measured in mol L⁻¹). n.d.: not determined.

the substitution of the thiazole ring does not change significantly the pK_{aH} (<0.5 log) of the biamidine, but it has a progressive effect on the $\log D$ (Me < diMe < *m*-NO₂Ph < Ph, see ESI[†]). Finally, we correlated these predictions with the experimental determination of pK_{aH} for compounds B1 and 7, with guanidine

and *o*-tolylbiguanide as reference compounds (Table 1). The experimental values were in accordance with the predictions, with measured $pK_{\text{aH}}(\mathbf{B1}) = 7.00 \pm 0.02$.

The effect of the thiazolyl moiety on the structure of the biamidine core was studied by crystal structure analysis of derivative **B1** (Fig. 3a). Surprisingly, the biamidine core proved to appear as the tautomer with two NH_2 groups, contrary to the representation in previous reports.^{8–11} This is supported by infrared analysis, which shows a strong absorption band at 1652 cm^{-1} (see ESI[†]), assigned to the out-of-phase deformation planar mode of the NH_2 group (1660 cm^{-1} for guanidine NH_2),¹⁶ and correlated by ^1H - ^1H NOESY NMR experiments (see ESI[†]). Moreover, the molecular conformation of the studied compounds is stabilized by two strong intramolecular hydrogen bonds $\text{N3-H3A} \cdots \text{N5}$ of 205 pm and $\text{N1-H1A} \cdots \text{N4}$ of 199 pm (Fig. 3a).

The ^1H NMR shifts corresponding to the NH_2 are represented as four characteristic peaks largely split over the range around 5–12 ppm. This pattern involving two sharp and two broad peaks corresponding to individual NH_2 groups is found in all thiazolyl biamidine derivatives and represent a clear signature of this family of compounds. Dynamic NMR experiments at different temperatures in $\text{DMSO-}d_6$ were performed using **B1** as model to compare the strength of the H-bonding in solution (Fig. 3b). The two sharp peaks appearing more downfield correspond to the unshielded $\text{H}_{\text{a,b}}$ atoms with H_{a} involved in intramolecular H-bonding (10.5 ppm). ^1H NMR signals of $\text{H}_{\text{c,d}}$ coalesce at $62\text{ }^\circ\text{C}$ while the other NH_2 displays split signals, demonstrating the faster rotation speed of C8–N3 bond and lower energy barrier for the corresponding H-bond. At $86\text{ }^\circ\text{C}$, we observe the merge of both N–H peaks into a single one for each NH_2 group, implying the fast exchange of both hydrogen atoms in H-bonds. Yet, additional experiments were carried out in $\text{CDCl}_3/\text{CD}_3\text{OD}$ mixtures at $25\text{ }^\circ\text{C}$ to show the faster deuterium exchange for the weaker H-bond. At fixed time (10 min), the addition of 5% of protic deuterated solvent promotes a deuterium exchange with the most accessible protons due to faster rotation and exchange

of NH_cH_d group, rather than with the protons involved in stronger hydrogen bond $\text{N1-H1A} \cdots \text{N4}$, as demonstrated by the disappearance of the corresponding signals from the spectrum (Fig. 3c).

In summary, the thiazolyl biamidine derivatives show strong double intramolecular H-bonding that freezes their conformation and explains their high solubility in apolar solvents. Besides, 2-pyridyl derivatives (**A10**, **B7**, **C7**, **D6**) seem to show an additional H-bond between the pyridyl N and H_{ab} , as supported by sharper NMR H_{ab} peaks and higher apolarity than the respective 3- and 4-pyridyl analogues (see ESI[†]). More generally, these features help reducing the polarity and flexibility of the biamidine function and can improve the physicochemical characteristics of biamidines towards druglikeness criteria.

We evaluated the biological activity of the 37 biamidine derivatives on the viability of metastatic melanoma cells, a cancer known for its high resistance to the current targeted therapies.¹⁷ The rationale for this model relies on the fact that antimelanoma activity has been recently described for biguanide drugs.^{18–20} Nevertheless, the high polarity and flexibility related to these compounds lead to reduced efficacy.²¹ Thus, our arylbiamidines can be considered as biguanide analogues, with reduced polarity and flexibility. Hence, the cell viability of all compounds (at $5\text{ }\mu\text{M}$ and $10\text{ }\mu\text{M}$) was evaluated on A375 human aggressive melanoma cells after treatment for 24 h and 48 h (see ESI[†]). Overall, dimethylthiazolyl derivatives from group B showed the highest activity among all groups ($<50\%$ at $10\text{ }\mu\text{M}$ after 48 h, Fig. 4a), with compound **B6** being the most active at any of the conditions tested (see ESI[†]), although compound **B7** showed similar results but only at $10\text{ }\mu\text{M}$ after 48 h. Comparison to PLX-4032, the current first line treatment for cutaneous melanoma patients carrying B-RAFV600 mutations,^{22,23} demonstrated a clearly higher activity at 2.5 and $5\text{ }\mu\text{M}$ concentrations (Fig. 4b, see ESI[†]), confirming **B6** efficacy. Moreover, we identified apoptosis as type of cell death occurring after **B6** treatment, as assessed by the cleavage of PARP protein (Fig. 4c).

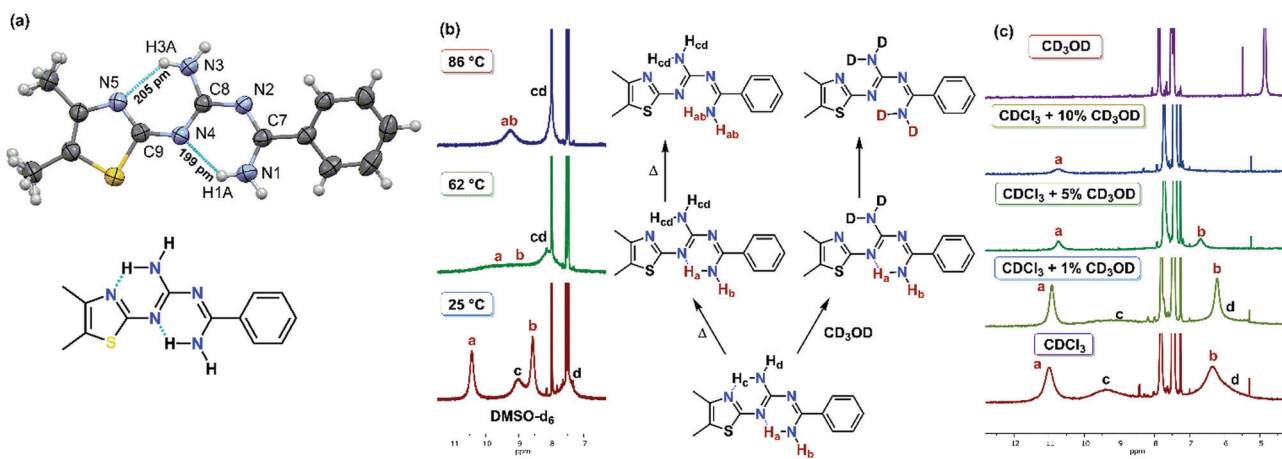


Fig. 3 Tautomerism and fine structure of **B1**. (a) View of the structure of the crystals of **B1**. Hydrogen atoms appear in white, carbon in grey, nitrogen in blue and sulphur in yellow. (b) Effect of the temperature on the ^1H NMR shifts of NH_2 . (c) Effect of deuterium exchange with increasing amounts of CD_3OD .

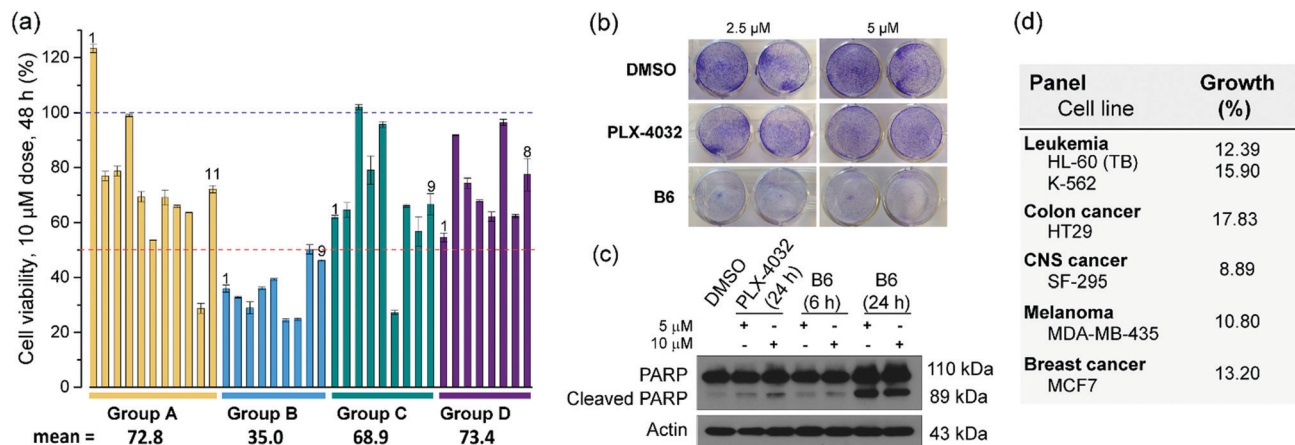


Fig. 4 Biological evaluation of diarylbiamidines. (a) Cell viability of A375 melanoma cells relative to DMSO upon treatment of 10 μM of the arylbiamidine derivatives of groups **A**, **B**, **C** and **D** at 48 h. Values are shown in the ESI†. Blue trace indicates the EC₅₀. $N = 3 \pm SD$. Mean is the average between all values within each group. (b) Cell viability assessed with crystal violet staining of A375 melanoma cells incubated for 24 h with DMSO (negative control), PLX-4032 or **B6**. (c) Western blotting analysis of apoptosis related protein PARP from A375 melanoma cells incubated for 24 h with DMSO, **B6** or PLX-4032 (actin is used as loading control). (d) Selected *in vitro* tumour growth data (<20%) for compound **B6**. Data obtained from the NCI's *in vitro* disease-oriented human tumour cells screening against 60-cancer cell lines. Results of the complete study are presented in the ESI†.

Compound **B6** was subsequently evaluated on a panel of 60 other cancer cell lines through the DTP program of the National Cancer Institute.²⁴ This screening led to cancer cell growth lower than 50% in 28 cancer cell lines (see ESI†) and lower than 20% in 6 cancer cell lines (Fig. 4d). The best results were obtained against CNS cancer and melanoma, specifically on the SF-295 and MDA-MB-435 cell lines, respectively.

Based on these results, we performed an *in silico* study to assess the druglikeness of compound **B6**.²⁵ As a result, it showed compatibility with the main druglike criteria (Lipinski, Ghose, Veber, Egan, Muegge, see ESI†). Taken together, these results strongly suggest anticancerous effects of compound **B6** on melanoma cells and, overall, confirm the pertinence of our designed molecules of group B as promising anticancer scaffold to be optimized.

In conclusion, we designed a novel family of biamidine derivatives containing one thiazolyl and one aryl moiety. A simple, robust and high-yielding synthetic route was developed for the synthesis of 37 derivatives. The study of the fine molecular structure of the dimethylthiazolyl biamidine compound **B1** by X-ray crystal analysis, IR and NMR experiments revealed the existence of a single tautomer with a strong double intramolecular H-bonding network, which deeply affects the polarity, solubility and stability properties of this family of compounds. Moreover, we determined their weak basicity ($pK_{aH} \approx 7$). The biological evaluation of all compounds on a metastatic melanoma model showed a structure activity relationship favourable for dimethylthiazolyl derivatives (group B). The most active compound **B6** demonstrated superior activity compared to reference standard drug PLX-4032, and activity towards other cancer cell lines. Overall, the peculiar structure of these arylbiamidine derivatives provides attractive physicochemical properties that might influence their biological activity. These results support the development of new arylbiamidine analogues as anticancer compounds and highlight the applicability of this non-exploited functional group in drug discovery.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was supported by the Cancéropôle PACA and the SATT-Sud Est (EmA grant). Université Côte d'Azur (IDEX UCA-JEDI Innovation Program), CNRS, ANR and INCA are also acknowledged for additional funding. The authors thank Dr Michel Giorgi for X-ray crystallography and Prof. Erick L. Bastos for constructive discussions.

Notes and references

- D. Kathuria, A. A. Bankar and P. V. Bharatam, "What's in a structure?" The story of biguanides, *J. Mol. Struct.*, 2018, **1152**, 61–78.
- J. Y. Quek, T. P. Davis and A. B. Lowe, Amidine functionality as a stimulus-responsive building block, *Chem. Soc. Rev.*, 2013, **42**(17), 7326–7334.
- J. E. Taylor, S. D. Bull and J. M. Williams, Amidines, isothioureas, and guanidines as nucleophilic catalysts, *Chem. Soc. Rev.*, 2012, **41**(6), 2109–2121.
- F. Sączewski and Ł. Balewski, Biological activities of guanidine compounds, 2008–2012 update, *Expert Opin. Ther. Pat.*, 2013, **23**(8), 965–995.
- R. G. S. Berlinck and S. Romminger, The chemistry and biology of guanidine natural products, *Nat. Prod. Rep.*, 2016, **33**(3), 456–490.
- M. Pollak, Potential applications for biguanides in oncology, *J. Clin. Invest.*, 2013, **123**(9), 3693–3700.
- O. Grytsai, C. Ronco and R. Benhida, Synthetic accesses to biguanide compounds, *Beilstein J. Org. Chem.*, 2021, **17**, 1001–1040.

- 8 S. Birtwell, F. Curd and F. Rose, Synthetic antimalarials. Part XLIV. The preparation of diguanides by the reaction of substituted amino-(including guanidino-) magnesium halides with the $>N\cdot C$ [triple bond, length as m-dash] N group, *J. Chem. Soc.*, 1949, 2556–2561.
- 9 E. Haruki, T. Inaike and E. Imoto, Some Reactions of N-Haloamidines, *Bull. Chem. Soc. Jpn.*, 1968, **41**(6), 1361–1367.
- 10 D. W. Ludovici, M. J. Kukla, P. G. Grous, S. Krishnan, K. Andries, M.-P. de Bethune, H. Azijn, R. Pauwels, E. De Clercq and E. Arnold, Evolution of anti-HIV drug candidates. Part 1: From α -anilinophenylacetamide (α -APA) to imidoyl thiourea (ITU), *Bioorg. Med. Chem. Lett.*, 2001, **11**(17), 2225–2228.
- 11 R. J. Pietras, M. E. Jung, D. C. Marquez-Garban and G. Deng, *Compounds and methods of treating cancer*, WO2013188452, 2013.
- 12 M. S. Al-Saadi, H. M. Faidallah and S. A. Rostom, Synthesis and biological evaluation of some 2,4,5-trisubstituted thiazole derivatives as potential antimicrobial and anti-cancer agents, *Arch. Pharm.*, 2008, **341**(7), 424–434.
- 13 S. M. Cascioferro, B. Parrino, D. Carbone, D. Schillaci, E. Giovannetti, G. Cirrincione and P. Diana, Thiazoles, their benzofused systems and thiazolidinone derivatives: versatile and promising tools to combat antibiotic resistance, *J. Med. Chem.*, 2020, **63**(15), 7923–7956.
- 14 P. Arora, R. Narang, S. K. Nayak, S. K. Singh and V. Judge, 2, 4-Disubstituted thiazoles as multitargeted bioactive molecules, *Med. Chem. Res.*, 2016, **25**(9), 1717–1743.
- 15 A. Rouf and C. Tanyeli, Bioactive thiazole and benzothiazole derivatives, *Eur. J. Med. Chem.*, 2015, **97**, 911–927.
- 16 W. J. Jones, The infra-red spectrum and structure of guanidine, *Trans. Faraday Soc.*, 1959, **55**, 524–531.
- 17 A. Millet, A. R. Martin, C. Ronco, S. Rocchi and R. Benhida, Metastatic melanoma: insights into the evolution of the treatments and future challenges, *Med. Res. Rev.*, 2017, **37**(1), 98–148.
- 18 M. Cerezo, M. Tichet, P. Abbe, M. Ohanna, A. Lehraiki, F. Rouaud, M. Allegra, D. Giaccherio, P. Bahadoran and C. Bertolotto, Metformin blocks melanoma invasion and metastasis development in AMPK/p53-dependent manner, *Mol. Cancer Ther.*, 2013, **12**(8), 1605–1615.
- 19 M. Cerezo, T. Tomic, R. Ballotti and S. Rocchi, Is it time to test biguanide metformin in the treatment of melanoma?, *Pigm. Cell Melanoma Res.*, 2015, **28**(1), 8–20.
- 20 T. Tomic, T. Botton, M. Cerezo, G. Robert, F. Luciano, A. Puissant, P. Gounon, M. Allegra, C. Bertolotto and J. Bereder, Metformin inhibits melanoma development through autophagy and apoptosis mechanisms, *Cell Death Dis.*, 2011, **2**(9), e199.
- 21 E. Jaune, E. Cavazza, C. Ronco, O. Grytsai, P. Abbe, N. Tekaya, M. Zerhouni, G. Beranger, L. Kaminski, F. Bost, M. Gesson, M. Tulic, P. Hofman, R. Ballotti, T. Passeron, T. Botton, R. Benhida and S. Rocchi, Discovery of a new molecule inducing melanoma cell death: dual AMPK/MELK targeting for novel melanoma therapies, *Cell Death Dis.*, 2021, **12**(1), 64.
- 22 A. Millet, M. Plaisant, C. Ronco, M. L. Cerezo, P. Abbe, E. Jaune, E. Cavazza, S. P. Rocchi and R. Benhida, Discovery and Optimization of *N*-(4-(3-Aminophenyl)thiazol-2-yl)acetamide as a Novel Scaffold Active against Sensitive and Resistant Cancer Cells, *J. Med. Chem.*, 2016, **59**(18), 8276–8292.
- 23 E. Hodis, R. Ian Watson, V. Gregory Kryukov, T. Stefan Arold, M. Imielinski, J.-P. Theurillat, E. Nickerson, D. Auclair, L. Li, C. Place, D. DiCara, A. H. Ramos, S. Michael Lawrence, K. Cibulskis, A. Sivachenko, D. Voet, G. Saksena, N. Stransky, C. Robert Onofrio, W. Winckler, K. Ardlie, N. Wagle, J. Wargo, K. Chong, L. Donald Morton, K. Stemke-Hale, G. Chen, M. Noble, M. Meyerson, E. John Ladbury, A. Michael Davies, E. Jeffrey Gershenwald, N. Stephan Wagner, S. B. Dave Hoon, D. Schadendorf, S. Eric Lander, B. Stacey Gabriel, G. Getz, A. Levi Garraway and L. Chin, A Landscape of Driver Mutations in Melanoma, *Cell*, 2012, **150**(2), 251–263.
- 24 A. Monks, D. Scudiero, P. Skehan, R. Shoemaker, K. Paull, D. Vistica, C. Hose, J. Langley, P. Cronise, A. Vaigro-Wolff, M. Gray-Goodrich, H. Campbell, J. Mayo and M. Boyd, Feasibility of a High-Flux Anticancer Drug Screen Using a Diverse Panel of Cultured Human Tumor Cell Lines, *JNCI, J. Natl. Cancer Inst.*, 1991, **83**(11), 757–766.
- 25 A. Daina, O. Michielin and V. Zoete, SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules, *Sci. Rep.*, 2017, **7**(1), 42717.