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Original article

Synthesis, in vitro antiplatelet activity and molecular modelling studies of 10-substituted 2-(1-piperazinyl)pyrimido[1,2-*a*]benzimidazol-4(10*H*)-ones

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ABSTRACT

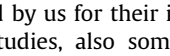
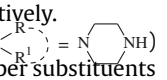
The multistep preparation of the new 10-substituted 2-(1-piperazinyl)pyrimido[1,2-*a*]benzimidazol-4(10*H*)-ones **6a**–**o**, and of the two isomers 10-ethyl-2-(diethylamino)pyrimido[1,2-*a*]benzimidazol-4(10*H*)-one **6p** and 10-ethyl-4-(diethylamino)pyrimido[1,2-*a*]benzimidazol-2(10*H*)-one **13**, as well as the in vitro evaluation of their inhibitory activity on human platelet aggregation induced in platelet-rich plasma by ADP, collagen or the Ca^{2+} ionophore A23187 were here described. Nine out of fifteen 2-(1-piperazinyl)derivatives (**6g**–**o**) showed good inhibitory properties towards all the platelet aggregation agonists used. Moreover, a molecular modelling study has been performed on two of the best compounds of this series (**6i** and **6o**) to confirm in silico their interactions with the catalytic site of human platelet PDE3, using the X-ray data of the PDE3B isoform in complex with an inhibitor.

1. Introduction

Since long time we have been interested in the synthesis of novel heterocyclic compounds endowed with human platelet aggregation in vitro inhibitory properties. In the course of our studies we synthesized and tested for their in vitro antiplatelet activity a number of substituted 2-aminochromones **1** [1–3], 4-aminocoumarins **2** [2–5] and their angular and linear benzo-fused derivatives [2,3]. Also several *N*-substituted 2-amino-4*H*-pyrido[1,2-*a*]pyrimidin-4-ones **3** [3,6–8], and 4-amino-2*H*-pyrido[1,2-*a*]pyrimidin-2-ones **4** [7], isosteric analogues of compounds **1** and **2**, respectively, and their benzo-fused angular derivatives [7] were synthesized and evaluated by us for their in vitro antiplatelet properties. Within these studies, also some other nitrogen bridgehead compounds **5**, differing from **3** for proper modifications of the pyridine ring, were prepared and tested [3,8] (Fig. 1).

Furthermore, it must be pointed out that also several 7-substituted or 7,8-disubstituted 2-(dialkylamino)-4*H*-1,3-benzoxazin-4-ones, isosteric analogues of the substituted 2-aminochromones **1**, have been reported as highly effective in vitro antiplatelet agents [9,10].

The antiplatelet activity data of the above-mentioned compounds **1**–**5** towards all the platelet aggregation agonists used [i.e. adenosine diphosphate (ADP), collagen and the Ca^{2+} ionophore A23187 (calcimycin)] allow us to draw the following conclusions:

- The bicyclic compounds **1**–**4** were more active than their linear and angular benzo-fused derivatives.
- In each structural class, the 1-piperazinyl group was the most effective amino substituent among all those tested, even though such a substitution () was not possible in the case of 4-amino-2*H*-pyrido[1,2-*a*]pyrimidin-2-ones **4** and their benzo-fused derivatives [7,8].
- The pyrido[1,2-*a*]pyrimidine derivatives **3** and, specially, the coumarins **2** were more effective in vitro antiplatelet agents in comparison with their isomers **4** and **1**, respectively.
- The coumarin scaffold of compounds **2** () appeared particularly suitable for inserting proper substituents in positions 7 and 8, in order to increase the antiplatelet activity potency. In fact, compounds **2a**, **2b** and **2c** (Fig. 2), obtained following this rationale, were the most potent in vitro antiplatelet agents synthesized by us [4,5].

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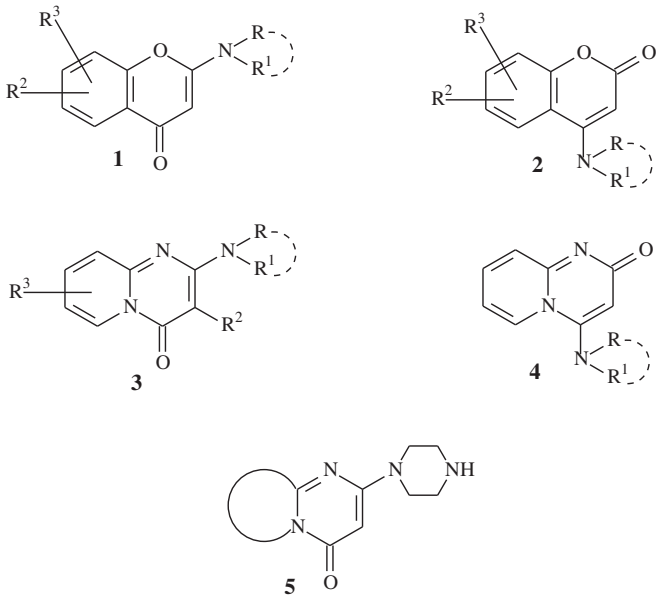


Fig. 1. Structures of compounds **1**–**5**.

- In regard to the mechanism involved in the antiplatelet activity of compounds **1**–**5**, it is interesting to point out that 2-(diethylamino)-7-hydroxychromone **1a** [11], the 2-(1-piperazinyl)-4*H*-pyrido[1,2-*a*]pyrimidin-4-one **3a** [12] as well as the coumarin derivative **2a** [13] (Fig. 2) proved to exert their platelet antiaggregating effect by specifically inhibiting the activity of the platelet PDE3 enzyme, producing an increase in the intracellular cAMP concentration. This finding confirmed the expected bioisosterism of the benzopyran derivatives **1**, **2** and 1,2-fused pyrimidine derivatives **3**–**5**. Moreover, it was shown that the coumarin derivative **2a** was able to increase nitric oxide formation [13].
- The substituted 4-(1-piperazinyl)coumarins **2a** and **2c** (Fig. 2) displayed a very potent inhibitory effect on the activity of PDE3 isolated from human platelets (IC_{50} values = 37 nM and 78 nM, respectively), better than that of milrinone (IC_{50} = 280 nM) and cilostazol (IC_{50} = 210 nM) [5].

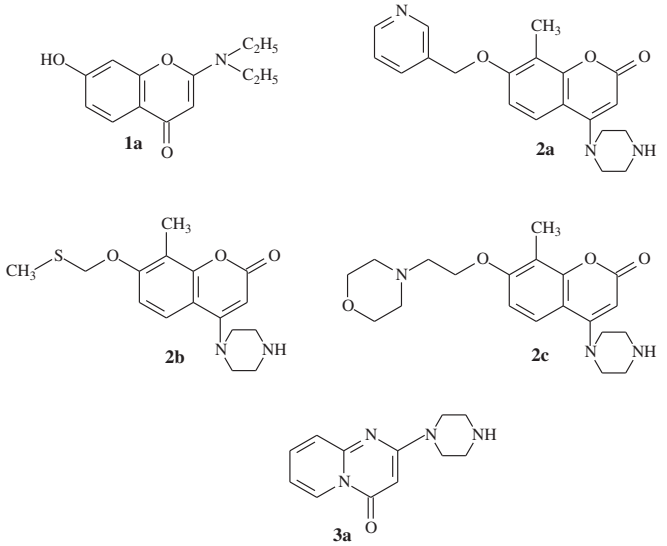


Fig. 2. Structures of compounds **1a**, **2a**–**c** and **3a**.

Starting from these results, we have pursued our studies to obtain new potent antiplatelet agents also in the field of 1,2-fused pyrimidine derivatives. Our interest was attracted by the structure of the 10-substituted 2-(1-piperazinyl)pyrimido[1,2-*a*]benzimidazol-4(10*H*)-ones **6**. In fact, even though generally, in each structural class studied, the bicyclic compounds **1**–**4** were more active than their benzofused analogues, some tricyclic nitrogen bridgehead compound such as the 7-chloro-2-(1-piperazinyl)-4*H*-pyrimido[2,1-*b*]benzoxazol-4-one (**5a**), which structure is analogous to compounds **6**, had previously shown very interesting in vitro antiplatelet properties [3]. Moreover, compounds **6**, bearing a NH group in position 10 were suitable for preparing a series of new promising derivatives by attaching in this position several proper substituents, preferring those that had given the best results when they were inserted on the coumarin scaffold of compounds **2**. (Fig. 3).

Therefore, in this paper we report the synthesis of a series of 10-substituted 2-(1-piperazinyl)-pyrimido[1,2-*a*]benzimidazol-4(10*H*)-ones **6a**–**o** and their antiplatelet activity. It must be noted that until now, no dialkylamino derivative of pyrimido[1,2-*a*]benzimidazol-4(10*H*)-one has been already described in the literature.

2. Chemistry

The synthetic route to compounds **6a**–**o** is shown in Scheme 1. Thus, the reaction of 2-aminobenzimidazole (**7**) with proper alkyl halides in refluxing acetone in the presence of anhydrous K_2CO_3/KOH gave good yields of 1-substituted 2-aminobenzimidazoles **8a**–**f**,**l**–**o** (Method A). On the contrary, in the case of compounds **8g**–**k** this method was not satisfactory. Therefore, in these cases, 2-aminobenzimidazole (**7**) was treated with an equimolar amount of EtONa in anhydrous EtOH to give the sodium salt derivative; after removal of solvent, this salt was taken up in acetone and treated with an equimolar amount of the proper alkyl halide, refluxing the mixture for 30 min to obtain the desired compounds **8g**–**k** (Method B). The 1-substituted 2-aminobenzimidazoles **8a**–**o** were then condensed with diethyl malonate (excess 10:1) in the presence of EtONa (2.5 eq) (150 °C, 3 h) to afford good yields of crystalline sodium salts of compounds **9a**–**o**. The nearly pure 10-substituted 2-hydroxypyrimido[1,2-*a*]benzimidazol-4(10*H*)-ones **9a**–**o** (most likely as zwitterionic structure in solid state [14–16]) were then obtained, as white solids, after a suitable acidification of the aqueous solution of these salts (compounds **9l**–**o**, bearing a tertiary amino group on the side chain, were precipitated as water-insoluble perchlorates). The subsequent treatment of **9a**–**o** with excess refluxing $POCl_3$ (2 h) afforded the corresponding 2-chloroderivatives **10a**–**o**, in satisfactory yields (35–93%, average 59%). The last step of the synthesis was the reaction of 2-chloroderivatives **10a**–**o** with excess piperazine in refluxing ethanol (3 h) to give usually good yields (52–92%, average 71%) of the desired 10-substituted 2-(1-piperazinyl)pyrimido[1,2-a]benzimidazol-4(10*H*)-ones **6a**–**o**.

It must be pointed out that the critical step of this synthetic route was the preparation of 2-hydroxyderivatives **9a**–**o**. Our first

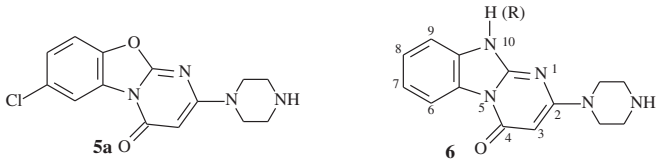
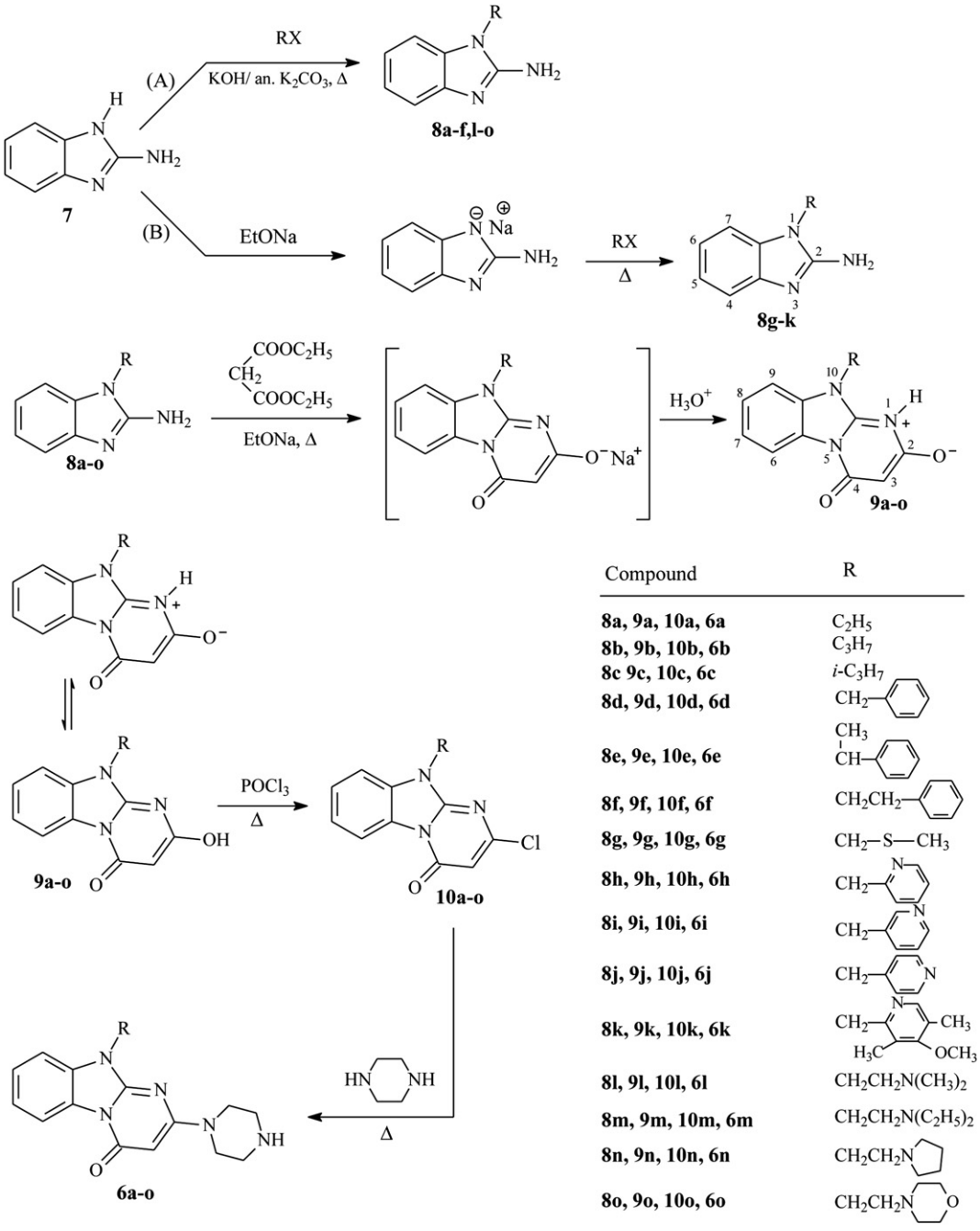


Fig. 3. Structural analogy between compound **5a** [3] and the 10-substituted 2-(1-piperazinyl)pyrimido[1,2-*a*]benzimidazol-4(10*H*)-ones **6**.

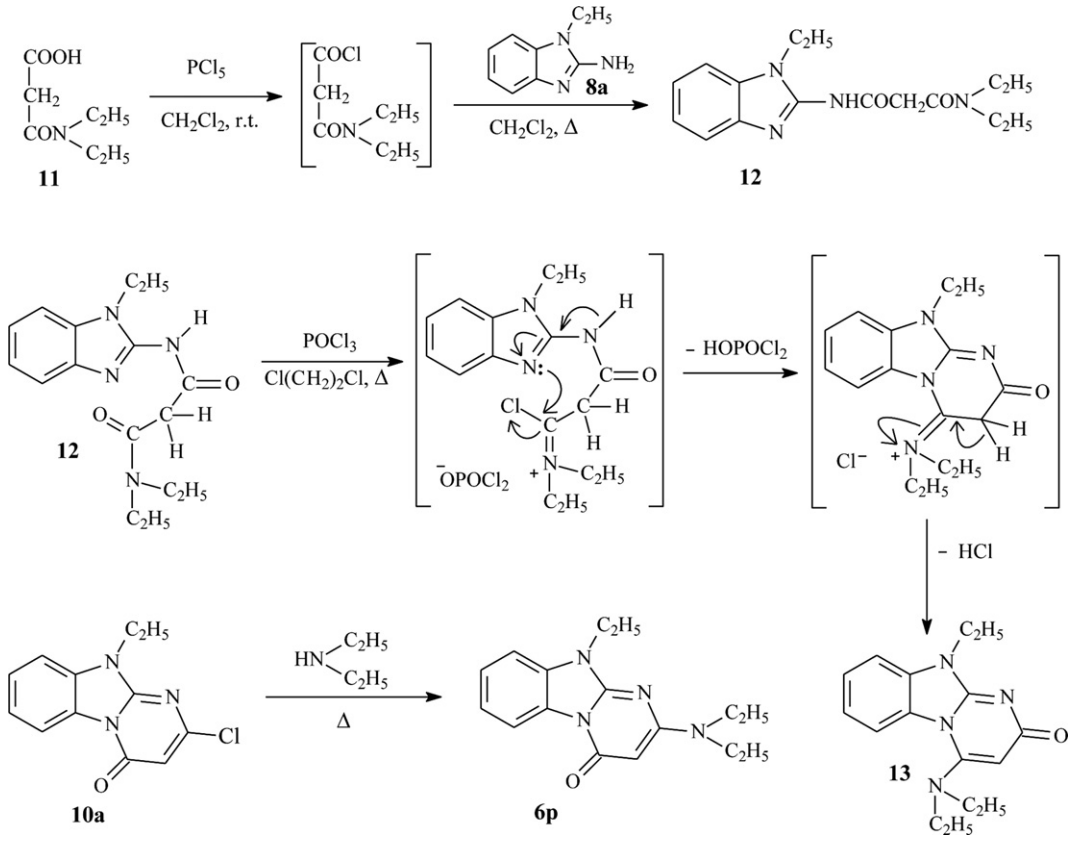


Scheme 1. Synthetic route to compounds **6a**–**o**.

attempts using the malonamate approach of Hermecz et al. [17], or the di(2,4,6-trichlorophenyl) malonate method [18,3], gave satisfactory results only in few cases (compounds **9a**,**b**,**d**,**f**,**o**). However, checking the literature about this topic, our attention was attracted by a Japanese patent [19], that claimed the synthesis of a series of 3,10-disubstituted 4-hydroxypyrimido[1,2-*a*]benzimidazol-2(10*H*)-ones, through the condensation of properly 1-substituted 2-aminobenzimidazoles with 2-substituted diethyl malonates in the presence of EtONa. In contrast to the Japanese patent, we thought that the isomeric structure of 3,10-disubstituted 2-hydroxypyrimido[1,2-*a*]benzimidazol-4(10*H*)-ones was more likely for these compounds, in agreement with previous literature reports in the field of nitrogen bridgehead pyrimidine derivatives, which demonstrated that, when on the pyrimidine ring there are two carbonyl groups, the enolization always involves the carbonyl group far from the bridgehead nitrogen [14–16,20]. However, the reaction reported in the Japanese patent [19] was an excellent method to prepare the 10-substituted 2-hydroxypyrimido[1,2-a] benzimidazol-4(10*H*)-ones **9a**–**o** in very good yields (58–94%, average 78%).

In order to give a definitive chemical demonstration of the structure of compounds **6**, we prepared the 10-ethyl-4-(diethylamino)pyrimido[1,2-*a*]benzimidazol-2(10*H*)-one **13** through the univocal route reported in Scheme 2.

Thus, the one-pot reaction of *N*,*N*-diethylmalonamic acid **11** [7] with PCl_5 in CH_2Cl_2 (room temperature for 3 h) followed by condensation of the resulting acyl chloride with 1-ethyl-2-aminobenzimidazole (**8a**) (in refluxing CH_2Cl_2 for 1 h) afforded



Scheme 2. Synthesis of isomers **6p** and **13**.

the intermediate malonamide **12**, in moderate yield (31%). The subsequent cyclization of the malonamide **12** by treatment with $POCl_3$ (refluxing 1,2-dichloroethane, 1 h) afforded a satisfactory yield (56%) of the desired 10-ethyl-4-(diethylamino)pyrimido[1,2-*a*]benzimidazol-2(10*H*)-one **13**.

On the other hand, we prepared in very good yield (89%) the isomer **6p** through the standard method (excess diethylamine, refluxing ethanol, 16 h) starting from 2-chloroderivative **10a**. Since the structure of compound **13** is secure, due to its synthetic pathway, to isomer **6p** must be attributed the structure of 10-ethyl-2-(diethylamino)pyrimido[1,2-*a*]benzimidazol-4(10*H*)-one. In such way, also the structures of compounds **6a**–**o** (obtained through the same route) and of their intermediates **10a**–**o** and **9a**–**o** are validated.

Furthermore, the availability of **6p** and **13** allowed us to compare the antiplatelet activity of a pair of the two possible isomers of this new class of compounds, even though for this purpose it would have been better to compare two isomers bearing the 1-piperazinyl group. Unfortunately, the synthesis of the pharmacologically more interesting 4-(1-piperazinyl)pyrimido[1,2-*a*]benzimidazol-2(10*H*)-one isomers type-**13** was not possible. A similar problem was previously encountered also in the case of other nitrogen bridgehead compounds reported by us [7,8].

The structures attributed to the compounds described in this paper are supported by the results of elemental analyses, as well as by the IR and ^{1}H NMR spectral data (see Experimental protocols) which agree with the ones previously reported by us for analogous compounds [3,6–8].

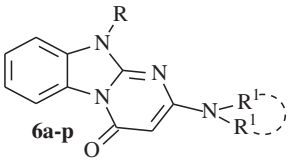
In the ^{1}H NMR spectra, particularly meaningful are the characteristic H-6 signals (multiplets with $J_{ortho} = 7.8$ Hz) of compounds **9**, **10** and **6**, shifted downfield due to deshielding effect of the nearly coplanar 4-CO. The chemical shift average values (δ) are: 8.44 (DMSO-d_6) for the 2-hydroxyderivatives **9**, 8.66 ($CDCl_3$) for the 2-chloroderivatives **10** and 8.56 ($CDCl_3$) for the 2-(1-piperazinyl)derivatives **6**, respectively. These data confirm that compounds **9**, **10** and **6** possess the same pyrimido[1,2-*a*]benzimidazol-4(10*H*)-one scaffold. Moreover, in the ^{1}H NMR spectrum of the 4-(diethylamino)-2-one derivative **13**, the H-6 signal is clearly less deshielded ($\delta = 7.92$) in comparison with the corresponding signal of the 2-(diethylamino)-4-one isomer **6p** ($\delta = 8.54$), according to a literature report for compounds belonging to the same heterocyclic system [21]. Another characteristic difference between the ^{1}H NMR spectra of the two isomers is the chemical shift of the pyrimidine ring proton (H-3), more deshielded in the 4-(diethylamino)-2-one derivative **13** ($\delta = 5.87$) in comparison with its 2-(diethylamino)-4-one isomer **6p** ($\delta = 5.19$). These spectroscopic features perfectly agree with those previously reported by us for analogous nitrogen bridgehead compounds [7].

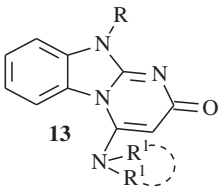
Moreover, for a further confirmation, a good number of representative examples of compounds **9**, **10** and **6** were subjected also to ^{13}C NMR spectrometry analysis and electrospray ionization mass spectrometry (ESI–MS) full scan and MS^2 analysis (see Experimental protocols). The results of these analyses are perfectly consistent with the proposed structures.

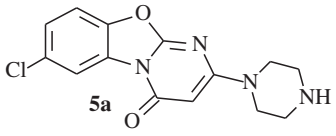
3. Biological results and discussion

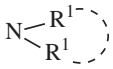
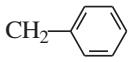
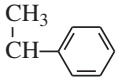
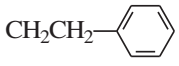
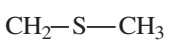
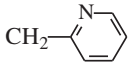
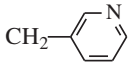
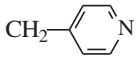
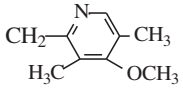
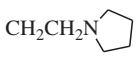
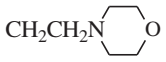
The seventeen novel compounds **6a**–**p** and **13** were tested in vitro for their inhibitory activity on the human platelet aggregation induced in platelet-rich plasma (PRP) by adenosine diphosphate (ADP), collagen, or the Ca^{2+} ionophore A23187 (calcimycin) (see Experimental protocols). Acetylsalicylic acid (ASA),

Table 1
In vitro inhibitory activity of the new pyrimido[1,2-*a*]benzimidazole derivatives **6a**–**p** and **13** on platelet aggregation induced in human PRP by ADP, collagen and A23187. The IC_{50} values previously described by us [3] for the pyrimido[2,1-*b*]benzoxazole derivative **5a** are herein reported for comparison.







Compound		R	IC_{50} (μM) ± SD[a]		
			ADP (5.0 μM)	Collagen (5.0 μg/mL)	A23187 (20.0 μM)
5a [3]	–	–	3.6 ± 1.2	8.8 ± 5.6	13.0 ± 5
6a		CH_2CH_3	127 ± 49	55 ± 28	206 ± 89
6b		$CH_2CH_2CH_3$	62 ± 13	50 ± 14	50 ± 8
6c			116 ± 17	77 ± 10	142 ± 38
6d			90 ± 20	27 ± 15	143 ± 27
6e			163 ± 11	172 ± 7	175 ± 12
6f			53 ± 3	15 ± 2	75 ± 21
6g			26 ± 3	31 ± 7	60 ± 15
6h			19 ± 1	30 ± 10	38 ± 7
6i			15 ± 6	9 ± 1	23 ± 13
6j			15 ± 6	21 ± 9	32 ± 9
6k			33 ± 10	49 ± 15	56 ± 18
6l		$CH_2CH_2N(CH_3)_2$	36 ± 5	8 ± 1	41 ± 4
6m		$CH_2CH_2N(C_2H_5)_2$	16 ± 7	13 ± 2	13 ± 5
6n			16 ± 2	28 ± 7	42 ± 11
6o			12 ± 5	15 ± 7	11 ± 6
6p	$N(C_2H_5)_2$	CH_2CH_3	>1000	75 ± 15	>1000
13	$N(C_2H_5)_2$	CH_2CH_3	>1000	75 ± 17	415 ± 150
ASA			>1000	183 ± 4	>1000
Cilostazol			73 ± 19	40 ± 9	109 ± 35
Milrinone			11 ± 4	12 ± 4	14 ± 5

[a] IC_{50} = compound concentration which inhibits platelet aggregation by 50%.

cilostazol and milrinone were also tested under the same conditions as reference compounds. The IC_{50} values obtained are reported in Table 1 along with the IC_{50} values previously described by us for the 7-chloro-2-(1-piperazinyl)-4*H*-pyrimido[2,1-*b*]benzoxazol-4-one (**5a**) [3], taken as a lead compound.

These data suggest the following remarks:

- Also in this class of 1,2-fused pyrimidine derivatives, the 1-piperazinyl group confirmed to be the best dialkylamino substituent, as shown by the comparison of IC_{50} values of **6a** and those of its diethylamino analogue **6p**.
- The 10-alkylsubstituted derivatives **6a**–**c** displayed a poor antiplatelet activity: among these three compounds, the highest activity was shown by the 10-propyl derivative **6b**, which performed better than the corresponding isopropyl analogue **6c**.
- Concerning, the 10-(arylalkyl)derivatives **6d**–**f**, it can be remarked that the substitution of the alkyl group with the benzyl one (**6d**) afforded only a modest antiplatelet activity, differently from what happened when this group was inserted on the 7-OH substituent of coumarins **2**. In fact, in that case we had obtained very interesting antiplatelet agents [3,4]. Slightly better results were afforded by the 10-(2-phenylethyl)derivative **6f**, whereas the branched chain derivative **6e** was the worst among these three compounds.
- The isosteric replacement of benzyl group with the three isomeric (pyridylmethyl)substituents afforded a small set of novel interesting antiplatelet agents (**6h**–**j**), with the (3-pyridylmethyl)derivative **6i** being the most active within these three compounds. It must be pointed out that the (3-pyridylmethyl)group was present also in the coumarin **2a** (Fig. 2), i.e. one of the most powerful antiplatelet agents previously synthesized by us [4,5]. Therefore, this result confirms the hypothesized bioisosterism between the 10-substituted 2-(1-piperazinyl)pyrimido[1,2-*a*]benzimidazol-4(10*H*)-ones **6** and the 7-substituted 4-(1-piperazinyl)coumarins **2**. When the pyridyl ring of the 10-substituent was hindered with further methyl and/or methoxy groups (compound **6k**), the antiplatelet potency decreased remaining anyway significant (Table 1).
- Also the introduction in 10-position of a proper 2-(dialkylamino)ethyl substituent revealed to be efficacious, leading to some very interesting compounds (**6l**–**o**) endowed with an antiplatelet potency comparable with that of (pyridylmethyl)derivatives **6h**–**j**. In particular the 2-(4-morpholinyl)ethylderivative **6o** was nearly equiactive with the (3-pyridylmethyl)derivative **6i**, showing a slightly lower effect against the aggregation induced by collagen, but a somewhat better efficacy against the aggregation induced by ADP and A23187. It's noteworthy that the 2-(4-morpholiny)ethyl substituent of compound **6o** was present also in the structure of the very active coumarin **2c** (Fig. 2) [5]. Moreover, also the 2-(diethylamino)ethyl derivative **6m** exhibited an antiplatelet activity very similar to those of compounds **6i** and **6o**.
- On the contrary, the introduction of a CH_2SCH_3 group in the position 10 of compounds **6** afforded compound **6g**, endowed with only a moderate antiplatelet activity (Table 1), while the analogous substitution was very efficacious when applied on the coumarin scaffold (compound **2b**, Fig. 2 [5]).

Finally, also the platelet antiaggregating properties of isomers **6p** and **13** were evaluated: both compounds were almost inactive, showing a mild activity only against the platelet aggregation induced by collagen (IC_{50} = 75 μM for both compounds). The keeping of a very poor activity against the platelet aggregation induced by A23187 (IC_{50} = 415 μM) by the 4-(diethylamino)-2-one isomer **13** was the only difference observed between the two isomers.

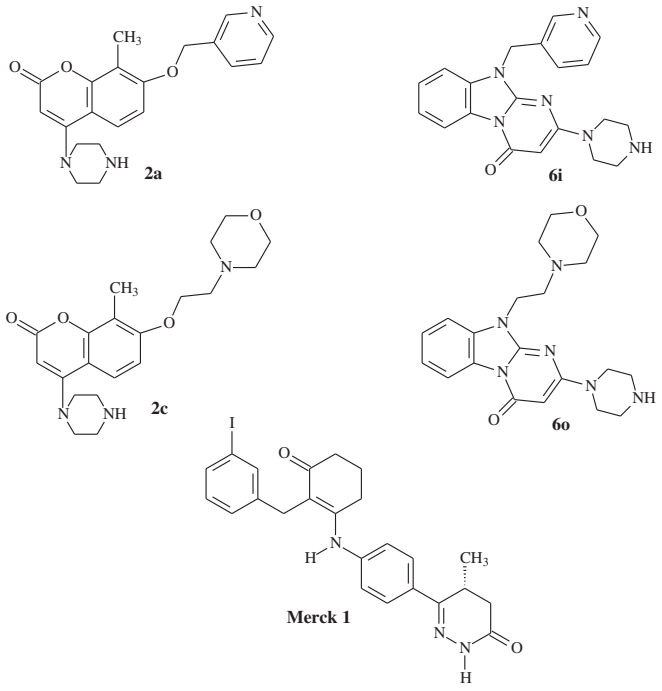


Fig. 4. Structures of compounds subjected to molecular modelling study.

On the basis of the structural analogies between the 10-substituted 2-(1-piperazinyl)pyrimido[1,2-*a*]benzimidazol-4(10*H*)-ones **6a**–**o** and the 4-(1-piperazinyl)coumarins **2** (Fig. 2) [5], it is very likely that also compounds **6a**–**o** could exert an inhibitory effect on the activity of human platelet PDE3.

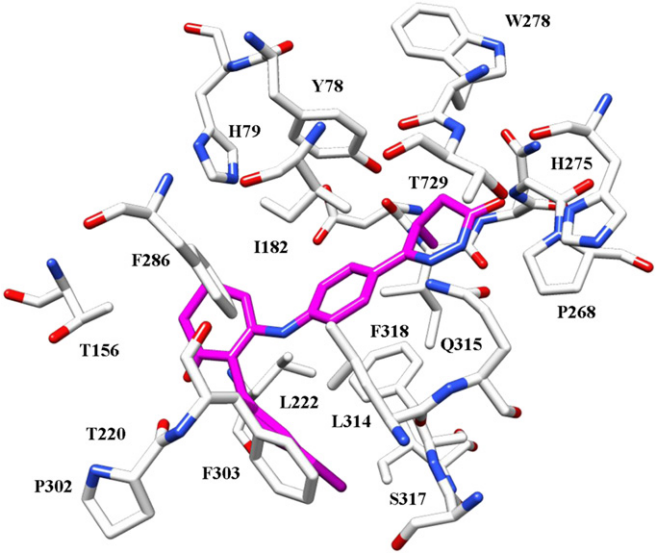


Fig. 5. The dihydropyridazinone inhibitor X-ray pose (pdb code 1SO2) into the PDE3B catalytic site is reported. The ligand *C* atoms are coloured in magenta. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

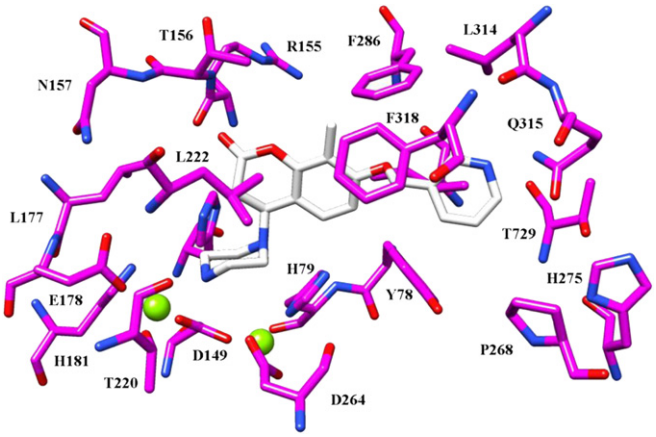


Fig. 6. Selected docking pose of compound **2a** into the PDE3B catalytic site (pdb code 1SO2). The ligand *C* atoms are coloured in white. Metal ions are represented as green spheres. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Molecular modelling studies

In order to confirm the hypothesized PDE3 inhibition by the compounds **6**, a molecular modelling study was performed on compounds **6i** and **6o**, chosen as examples, due to their high antiplatelet activity and their analogy to coumarin derivatives **2a** and **2c**, which were previously evaluated by us in silico for their ability to interact with the PDE3 catalytic site [5].

First of all, since compounds **2a** and **2c** proved to be very potent PDE3 inhibitors [5], they were submitted to docking studies using the X-ray data of the PDE3B isoform in complex with a dihydropyridazinone inhibitor [**Merck 1** (Fig. 4), Protein Data Bank (pdb) code 1SO2] [22]. On the basis of these results, compounds **6** were evaluated by us as potential PDE3 inhibitors: in particular we focused our attention on **6i** and **6o**, being the two compounds of this series more structurally related with the above mentioned **2a** and **2c**.

Thus, the **6i** and **6o** docking poses were compared with those of the two PDE3 inhibitors **2a** and **2c** in the 1SO2 complex (Fig. 4).

4.1. Discussion

In the 1SO2 X-ray pdb, the dihydropyridazinone inhibitor bioactive conformation (Fig. 5), displays H-bonds between: (*i*) one of the two nitrogen atoms of the dihydropyridazinone ring and the PDE3 residue Q315, (*ii*) the dihydropyridazinone ring oxygen atom and H275. The inhibitor is also engaged in π–π stacking with Y78, F286, and F318 through the aminophenyl group, and with the F303 residue, by means of the iodobenzyl ring. Furthermore, Van der Waals contacts with L222, I265, L314 and I322 are detected.

According to our molecular docking results, the PDE3 inhibitors **2a** (Fig. 6) and **2c** (Fig. 7) display one H-bond with the key residue Q315, through the pyridine and the morpholine ring, respectively, and are also engaged in π–π stacking with the Y78, F286 and F318 residues. In addition, the two compounds orient the piperazine ring towards the catalytic site metal ions, being also engaged in one H-bond with D149 side chain.

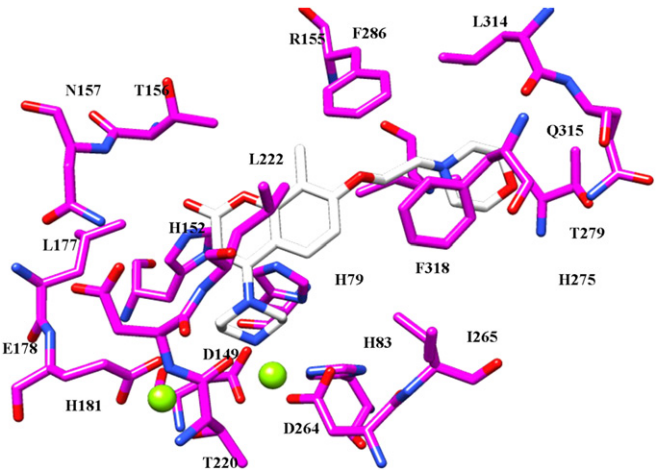


Fig. 7. Selected docking pose of compound **2c** into the PDE3B catalytic site (pdb code 1SO2). The ligand *C* atoms are coloured in white. Metal ions are represented as green spheres. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

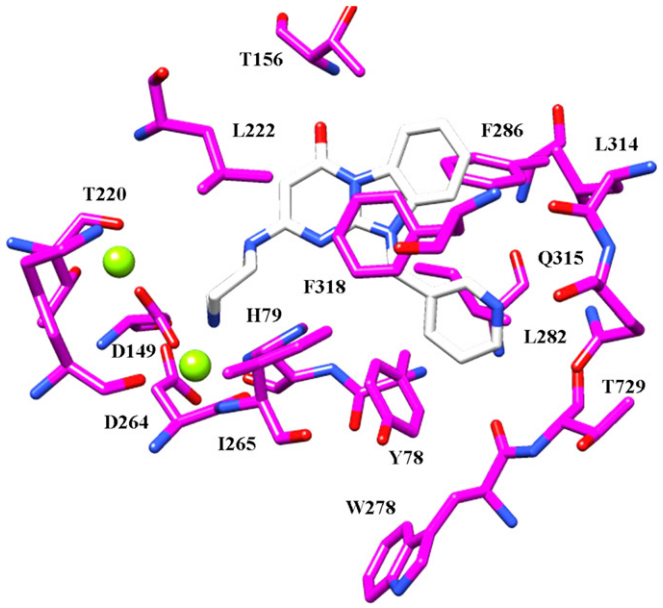


Fig. 8. Selected docking pose of compound **6i** into the PDE3B catalytic site (pdb code 1SO2). The ligand *C* atoms are coloured in white. Metal ions are represented as green spheres. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

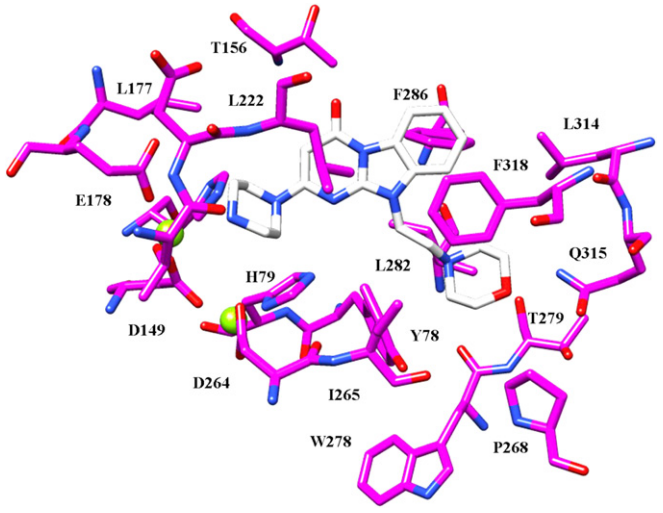


Fig. 9. Selected docking pose of compound **6o** into the PDE3B catalytic site (pdb code 1SO2). The ligand *C* atoms are coloured in white. Metal ions are represented as green spheres. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Interestingly, compounds **6i** (Fig. 8) and **6o** (Fig. 9) prove to show the same binding mode which has been previously observed for compounds **2a** and **2c**, respectively.

Thus, the **6i** pyridine ring and the **6o** morpholine one are involved in a H-bond with the key residue Q315, while the piperazine moiety is projected towards the enzyme metal ions and residue D149. In particular, the H-bond displayed by the **6i** pyridine ring seems to be slightly weaker (H-bond distance: 3.00 Å) than that of the **6o** morpholine ring (H-bond distance: 2.70 Å), probably because of the shorter linker which is located between the **6i** tricyclic ring and the pyridine group, in comparison with the linker placed between the **6o** scaffold and the morpholine moiety. Accordingly, compound **6o** displays an inhibitory activity on human platelet aggregation induced by ADP higher than that displayed by **6i**.

Notably, compounds **6** bearing no H-bond acceptor groups at the R substituent (compounds **6a**–**f**, Table 1) showed lower inhibitory activity values, being probably unable to act as PDE3 inhibitors, due to the absence of an anchoring H-bond with Q315 residue.

5. Conclusions

Taking into account the biological results of the new compounds **6a**–**p** and **13** described in the present paper, the following conclusions can be drawn:

- The 10-substituted 2-(1-piperazinyl)pyrimido[1,2-*a*]benzimidazol-4(10*H*)-ones **6a**–**o** proved to be a new interesting class of in vitro antiplatelet agents.
- Nine out of fifteen 2-(1-piperazinyl)derivatives **6a**–**o** (i.e. compounds **6g**–**o**) showed good inhibitory properties towards all the platelet aggregation inducers used; among these compounds, **6i**,**m**,**o** were active in the low micromolar range. As regards the in vitro antiplatelet efficacy, compounds **6i**,**m**,**o** were comparable with the 7-chloro-2-(1-piperazinyl)-4*H*-pyrimido[2,1-*b*]benzoxazol-4-one (**5a**) [3] and other (1-piperazinyl)substituted nitrogen bridgehead compounds, previously synthesized by us [3,7], even though inferior to the substituted 4-(1-piperazinyl)coumarins **2** [3–5].
- The (1-piperazinyl)substituent inserted in a β-enaminonic moiety confirmed to be a structural feature essential for displaying a good antiplatelet activity in this kind of compounds: in fact the two (diethylamino)substituted isomers **6p** and **13** were nearly inactive.
- A molecular modelling study showed that compounds **6i** and **6o** (two of the best compounds of the series) possess the same binding mode to the PDE3 catalytic site which has been already observed for the substituted 4-(1-piperazinyl)coumarins **2a** and **2c**, respectively. Since in a previous study compounds **2a** and **2c** proved to be very potent inhibitors of human platelet PDE3 [5], we can hypothesize that also compounds **6a**–**o** could exert their in vitro antiplatelet activity through the same mechanism of action.

6. Experimental protocols

6.1. Chemistry

Melting points were determined using a Fisher-Johns apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer "Spectrum One" spectrophotometer (abbreviations relative to IR bands: br = broad, s = strong, w = weak, sh = shoulder). ^{1}H NMR spectra were recorded partly on a Varian Gemini 200 (200 MHz) spectrometer and partly on a Bruker WM-300 (300 MHz) spectrometer; chemical shifts (δ) are reported in ppm using tetramethylsilane as an internal reference ($\delta = 0$). Spin multiplicities are given as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet). ^{13}C NMR spectra were acquired on a Bruker DPX spectrometer at 75.5 MHz with tetramethylsilane as internal reference. Electrospray ionization mass spectrometry (ESI–MS) full scan and MS^2 analysis were performed in positive or negative ion mode on a LCQ-Fleet ion trap mass spectrometer (ThermoFisher Scientific, UK) in direct infusion (DIA) using the infusion pump included in the instrument. The flow rate was set at 5 μL/min. Each sample was diluted in methanol at a concentration of 1 mg/mL and then diluted 100 fold in methanol:water 50:50 containing 0.1% formic acid to get a final concentration of 0.01 mg/mL. Analyses of all new compounds, indicated by the symbols of the elements, were within ±0.4% of the theoretical values and were performed by the Laboratorio di Microanalisi, Dipartimento di Scienze Farmaceutiche, University of Genoa.

Thin-layer chromatograms were run on Merck silica gel 60 F_{254} precoated plastic sheets (layer thickness 0.2 mm). Column chromatography was performed using Carlo Erba silica gel (0.05–0.20 mm).

6.1.1. General procedure for the synthesis of 1-substituted 1H-benzimidazol-2-amines *8a*–*o*

*6.1.1.1. Method A (compounds **8a**–**f**,**l**–**o**).* A mixture of 2-aminobenzimidazole **7** (1.33 g, 10.0 mmol), 10.0 mmol of the proper alkyl halide, 1.0 g of finely powdered KOH mixed with 2.0 g of anhydrous K_2CO_3 and acetone (50 mL) was heated at reflux for 3 h, with stirring. The solvent was removed in vacuo and the residue was partitioned between water (100 mL) and CH_2Cl_2 (100 mL), and the aqueous phase was further extracted twice with CH_2Cl_2. The combined extracts, dried over anhydrous Na_2SO_4, after removal of solvent afforded an oily or solid residue which was treated with a small amount of ethyl ether to give compounds **8a**–**e**,**l**–**o** as whitish solids which were crystallized from the proper solvent. Only in the case of compound **8f**, a preliminary purification by column chromatography [SiO_2/ethyl acetate-acetone (1:1)] was necessary to obtain a crystalline compound.

*6.1.1.2. Method B (compounds **8g**–**k**).* 2-Aminobenzimidazole **7** (1.33 g, 10.0 mmol) was dissolved in an ethanolic solution of sodium ethoxide [10.0 mmol (0.23 g) of sodium in 40 mL of anhydrous EtOH (preparation of **8g**) or 20.0 mmol (0.46 g) of sodium in 40 mL of anhydrous EtOH (preparation of **8h**–**k**), because in these cases the alkylating reagents were as hydrochlorides] and the mixture was evaporated to dryness in vacuo. The solid residue was suspended in acetone (50 mL) and treated with 10.0 mmol of the proper alkyl halide, refluxing the mixture for 30 min. The solvent was removed in vacuo and the residue was partitioned between water (100 mL) and CH_2Cl_2 (100 mL), and the aqueous phase was further extracted twice with CH_2Cl_2. The combined extracts, dried over anhydrous Na_2SO_4, after removal of solvent afforded a solid residue (compounds **8h**–**k**) which was treated with a small amount of ethyl ether to give compounds **8h**–**k** as whitish solids which were crystallized from the proper solvent. Only in the case of compound **8g**, was obtained an oil which was purified by column chromatography [SiO_2/ethyl acetate-acetone (1:1)] to give **8g** as a crystalline compound.

According to these two procedures the following compounds **8** were prepared (IR and ^{1}H NMR data are reported only for the new compounds and for **8h**,**i**,**n**, due to their incomplete literature data).

*6.1.1.3. 1-Ethyl-1H-benzimidazol-2-amine (**8a**).* Obtained (1.05 g, 65%) from reaction of **7** with iodoethane (1.56 g); white crystals,

m.p. 158–159 °C (diisopropyl ether) (lit. [23] m.p. 158–159 °C). Anal. $C_9H_{11}N_3$ (C, H, N).

*6.1.1.4. 1-Propyl-1H-benzimidazol-2-amine (**8b**).* Obtained (1.07 g, 61%) from reaction of **7** with 1-iodopropane (1.70 g); white crystals, m.p. 131–132 °C (diisopropyl ether) (lit. [23] m.p. 130 °C). Anal. $C_{10}H_{13}N_3$ (C, H, N).

*6.1.1.5. 1-Isopropyl-1H-benzimidazol-2-amine (**8c**).* Obtained (0.84 g, 48%) from reaction of **7** with 2-iodopropane (1.70 g); white crystals, m.p. 195–196 °C (EtOAc/petroleum ether) (lit. [24] m.p. 195–196 °C). Anal. $C_{10}H_{13}N_3$ (C, H, N).

*6.1.1.6. 1-Benzyl-1H-benzimidazol-2-amine (**8d**).* Obtained (1.72 g, 77%) from reaction of **7** with benzyl chloride (1.26 g); white crystals, m.p. 194–195 °C (EtOAc/petroleum ether) (lit. [23] m.p. 195–196 °C). Anal. $C_{14}H_{13}N_3$ (C, H, N).

*6.1.1.7. 1-(1-Phenylethyl)-1H-benzimidazol-2-amine (**8e**).* Obtained (1.74 g, 73%) from reaction of **7** with (1-bromoethyl) benzene (1.85 g); white crystals, m.p. 120–122 °C (EtOAc/petroleum ether) (lit. [25] m.p. 120–122 °C). Anal. $C_{15}H_{15}N_3$ (C, H, N).

*6.1.1.8. 1-(2-Phenylethyl)-1H-benzimidazol-2-amine (**8f**).* Obtained (0.71 g, 30%) from reaction of **7** with (2-bromoethyl) benzene (1.85 g); white crystals, m.p. 125–126 °C (Et_2O) (lit. [26] m.p. 120–122 °C). Anal. $C_{15}H_{15}N_3$ (C, H, N).

*6.1.1.9. 1-[(Methylthio)methyl]-1H-benzimidazol-2-amine (**8g**).* Obtained (0.60 g, 31%) from reaction of **7** with (chloromethyl) methyl sulphide (0.97 g); whitish crystals, m.p. 142–144 °C (EtOAc/petroleum ether). IR (KBr, cm^{-1}): 3322 br and 3110 br (NH_2), 1670, 1635, 1557 s, 1538. ^1H NMR (200 MHz, $CDCl_3$, ppm): δ 2.13 (s, 3H, NCH_2SCH_3), 3.95 (broad s, 2H, NH_2; disappeared with D_2O), 5.14 (s, 2H, NCH_2SCH_3), 7.07–7.53 (m, 4H, H-4,5,6,7). Anal. $C_9H_{11}N_3S$ (C, H, N, S).

*6.1.1.10. 1-(2-Pyridylmethyl)-1H-benzimidazol-2-amine (**8h**).* Obtained (1.51 g, 67%) from reaction of **7** with 2-(chloromethyl) pyridine hydrochloride (1.64 g); white crystals, m.p. 153–154 °C (EtOAc/petroleum ether) (lit. [27], no m.p. reported). IR (KBr, cm^{-1}): 3330 br and 3055 br (NH_2), 1660, 1615 w, 1596, 1571 sh, 1554 s. ^1H NMR (300 MHz, $CDCl_3$, ppm): δ 5.17 (s, 2H, NCH_2), 5.78 (broad s, 2H, NH_2; disappeared with D_2O), 7.03–7.27 and 7.42 (2 m, 5H + 1H, H-4,5,6,7 + pyridyl H-3′,5′), 7.65 (m, 1H, pyridyl H-4′), 8.56 (m, 1H, pyridyl H-6′). ^{13}C NMR (75.5 MHz, $CDCl_3$, ppm): δ 48.24, 107.09, 116.56, 119.73, 121.57, 122.31, 123.28, 134.10, 137.57, 142.09, 149.68, 154.84, 155.72. Anal. $C_{13}H_{12}N_4$ (C, H, N).

*6.1.1.11. 1-(3-Pyridylmethyl)-1H-benzimidazol-2-amine (**8i**).* Obtained (1.28 g, 57%) from reaction of **7** with 3-(chloromethyl) pyridine hydrochloride (1.64 g); white crystals, m.p. 247–248 °C (EtOAc) (lit. [27], no m.p. reported). IR (KBr, cm^{-1}): 3309 br and 3107 br (NH_2), 1672, 1618 w, 1595, 1578 sh, 1556 s. ^1H NMR (300 MHz, DMSO-d_6, ppm): δ 5.32 (s, 2H, NCH_2), 6.63 (s, 2H, NH_2; disappeared with D_2O), 6.82–6.98 (m, 2H, H-5,6), 7.10–7.18 (m, 2H, H-4,7), 7.34 (m, 1H, pyridyl H-5′), 7.55 (m, 1H, pyridyl H-4′), 8.46 (m, J_o = 4.8 Hz, J_m = 1.6 Hz, 1H, pyridyl H-6′), 8.51 (m, J_m = 1.6 Hz, 1H, pyridyl H-2′). Anal. $C_{13}H_{12}N_4$ (C, H, N).

*6.1.1.12. 1-(4-Pyridylmethyl)-1H-benzimidazol-2-amine (**8j**).* Obtained (0.88 g, 39%) from reaction of **7** with 4-(chloromethyl) pyridine hydrochloride (1.64 g); white crystals, m.p. 247–249 °C (acetone). IR (KBr, cm^{-1}): 3265 br and 3031 br (NH_2), 1672, 1618 w, 1605, 1554 s. ^1H NMR (200 MHz, DMSO-d_6, ppm): δ 5.33 (s, 2H, NCH_2), 6.58 (s, 2H, NH_2; disappeared with D_2O), 6.78–7.22 (m, 6H, H-4,5,6,7 + pyridyl H-3′,5′), 8.50 (m, 2H, pyridyl H-2′,6′). Anal. $C_{13}H_{12}N_4$ (C, H, N).

*6.1.1.13. 1-[(4-Methoxy-3,5-dimethyl-2-pyridyl)methyl]-1H-benzimidazol-2-amine (**8k**).* Obtained (1.79 g, 63%) from reaction of **7** with 2-(chloromethyl)-4-methoxy-3,5-dimethylpyridine hydrochloride (2.22 g); white crystals, m.p. 206–207 °C (EtOAc/petroleum ether). IR (KBr, cm^{-1}): 3390, 3341 and 3164 br (NH_2), 1669 s, 1619, 1594, 1570 w, 1547 s. ^1H NMR (200 MHz, $CDCl_3$, ppm): δ 2.27 and 2.44 (2 s, 3H + 3H, 2 CH_3), 3.76 (s, 3H, OCH_3), 5.20 (s, 2H, NCH_2), 5.93 (broad s, 2H, NH_2; disappeared with D_2O), 7.02–7.50 (m, 4H, H-4,5,6,7), 8.23 (s, 1H, pyridyl H-6′). Anal. $C_{16}H_{18}N_4O$ (C, H, N).

*6.1.1.14. 2-Amino-N,N-dimethyl-1H-benzimidazole-1-ethanamine (**8l**).* Obtained (1.23 g, 60%) from reaction of **7** with (2-chloroethyl) dimethylamine hydrochloride (1.44 g); white crystals, m.p. 147–149 °C (EtOAc/petroleum ether) (lit. [28] m.p. 140–142 °C). Anal. $C_{11}H_{16}N_4$ (C, H, N).

*6.1.1.15. 2-Amino-N,N-diethyl-1H-benzimidazole-1-ethanamine (**8m**).* Obtained (1.49 g, 64%) from reaction of **7** with (2-chloroethyl)diethylamine hydrochloride (1.72 g); white crystals, m.p. 134–135 °C (EtOAc/petroleum ether) (lit. [29] m.p. 134–135 °C). Anal. $C_{13}H_{20}N_4$ (C, H, N).

*6.1.1.16. 1-[2-(1-Pyrrolidinyl)ethyl]-1H-benzimidazol-2-amine (**8n**).* Obtained (1.64 g, 71%) from reaction of **7** with 1-(2-chloroethyl) pyrrolidine hydrochloride (1.70 g); white crystals, m.p. 172–173 °C (diisopropyl ether) (lit. [30], no m.p. reported). IR (KBr, cm^{-1}): 3321 br and 3134 br (NH_2), 1659 s, 1618 w, 1548 s. ^1H NMR (200 MHz, DMSO-d_6, ppm): δ 1.60–1.75 (m, 4H, pyrrolidinyl β-CH_2's), 2.44–2.57 (m, 4H, pyrrolidinyl N–CH_2's), 2.68 (t, 2H, NCH_2CH_2N–pyrrolidinyl), 4.75 (t, 2H, NCH_2CH_2N–pyrrolidinyl), 6.39 (s, 2H, NH_2; disappeared with D_2O), 6.78–6.99 (m, 2H, H-5,6), 7.10–7.15 (m, 2H, H-4,7). Anal. $C_{13}H_{18}N_4$ (C, H, N).

*6.1.1.17. 1-[2-(4-Morpholinyl)ethyl]-1H-benzimidazol-2-amine (**8o**).* Obtained (1.61 g, 65%) from reaction of **7** with 1-(2-chloroethyl) morpholine hydrochloride (1.86 g); white crystals, m.p. 190–191 °C (EtOAc) (lit. [29] m.p. 190–191 °C). Anal. $C_{13}H_{18}N_4O$ (C, H, N).

*6.1.2. General procedure for the synthesis of 10-substituted 2-hydroxypyrimido[1,2-a]benzimidazol-4(10H)-ones **9a**–**o***

Sodium (0.29 g, 12.5 mmol) was dissolved in 15 mL of anhydrous ethanol and this solution was concentrated to a small volume (2–3 mL), then 5.0 mmol of the proper 1-substituted 1*H*-benzimidazol-2-amine **8** and 50.0 mmol (8.00 g) of diethyl malonate were added and the resulting mixture was heated at 150 °C (under reflux) with stirring for 3 h. After cooling, the sodium salt of compound **9**, which precipitated as a whitish solid during the reaction, was collected, crushed in a mortar, suspended in Et_2O, filtered and dried. This solid was then dissolved in water and the resulting solution was acidified with an excess of 6 N aq HCl (or carefully acidified with 6 N aq HCl down to pH 4, in the case of the pyridyl derivatives compounds **9h**–**k**): this way compounds **9a**–**k** separated out as white or whitish solids that were collected by filtration, washed with water, dried and crystallized from the proper solvent. Only in the case of compounds **9l**–**o** (bearing a tertiary amino group on the side chain), the aqueous solution of sodium salt was treated with an excess of 70% aq. $HClO_4$ to get the precipitation of compounds **9l**–**o** as water-insoluble perchlorates which were collected by filtration, washed with water, dried and crystallized from ethanol. According to this procedure the following compounds **9** were obtained (IR and ^1H NMR data are reported only for the new compounds).

*6.1.2.1. 10-Ethyl-2-hydroxypyrimido[1,2-a]benzimidazol-4(10H)-one (**9a**).* Obtained (0.94 g, 82%) from **8a** (0.81 g); white crystals, m.p. 308–310 °C dec. (EtOH) (lit. [31] m.p. 281–283 °C). Anal. $C_{12}H_{11}N_3O_2$ (C, H, N).

*6.1.2.2. 2-Hydroxy-10-propylpyrimido[1,2-a]benzimidazol-4(10H)-one (**9b**).* Obtained (0.99 g, 81%) from **8b** (0.88 g); white crystals, m.p. 298 °C dec. (MeOH) (lit. [31] m.p. 253–255 °C). Anal. $C_{13}H_{13}N_3O_2$ (C, H, N).

*6.1.2.3. 2-Hydroxy-10-isopropylpyrimido[1,2-a]benzimidazol-4(10H)-one (**9c**).* Obtained (1.13 g, 93%) from **8c** (0.88 g); white crystals, m.p. 285–287 °C dec. (acetone). IR (KBr, cm^{-1}): 3180–2200 br (zwitterionic form NH^+), 1670 sh and 1631 (CO), 1603, 1558 s, 1529 w, 1496. ^1H NMR (300 MHz, DMSO-d_6, ppm): δ 1.62 and 1.68 [2d, J = 7 Hz, 3H + 3H, NCH(CH_3)$_2$], 5.11 [m, 1H, NC*H*(CH_3)$_2$], 5.17 (s, 1H, H-3), 7.34–7.49 (m, 2H, H-7,8), 7.79 (m, J_o = 8 Hz, 1H, H-9), 8.48 (m, J_o = 7.8 Hz, 1H, H-6), 11.35 (s, 1H, OH; disappeared with D_2O). Anal. $C_{13}H_{13}N_3O_2$ (C, H, N).

*6.1.2.4. 10-Benzyl-2-hydroxypyrimido[1,2-a]benzimidazol-4(10H)-one (**9d**).* Obtained (1.33 g, 91%) from **8d** (1.12 g); white crystals, m.p. 256–257 °C dec. (acetone) (lit. [19,32], no m.p. reported). Anal. $C_{17}H_{13}N_3O_2$ (C, H, N).

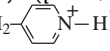
*6.1.2.5. 2-Hydroxy-10-(1-phenylethyl)pyrimido[1,2-a]benzimidazol-4(10H)-one (**9e**).* Obtained (1.03 g, 67%) from **8e** (1.19 g); white crystals, m.p. 232–234 °C dec. (EtOH/petroleum ether). IR (KBr, cm^{-1}): 3167–2378 br (zwitterionic form NH^+), 1671 s (CO), 1644 w, 1607, 1532 s. ^1H NMR (200 MHz, DMSO-d_6, ppm): δ 2.00 [d, J = 7 Hz, 3H, NCH(CH_3)C_6H_5], 5.23 (s, 1H, H-3), 6.22 [q, J = 7 Hz, 1H, NC*H*(CH_3)C_6H_5], 7.07–7.75 (m, 8H, H-7,8,9 + C_6H_5), 8.47 (m, J_o = 7.8 Hz, 1H, H-6), 11.45 (broad s, 1H, OH; disappeared with D_2O). Anal. $C_{18}H_{15}N_3O_2$ (C, H, N).

*6.1.2.6. 2-Hydroxy-10-(2-phenylethyl)pyrimido[1,2-a]benzimidazol-4(10H)-one (**9f**).* Obtained (1.09 g, 71%) from **8f** (1.19 g); white crystals, m.p. 260–261 °C dec. (MeOH/acetone). IR (KBr, cm^{-1}): 3170–2380 br (zwitterionic form NH^+), 1680 sh and 1632 (CO), 1610, 1556 s, 1500. ^1H NMR (300 MHz, DMSO-d_6, ppm): δ 3.12 (t, J = 7.3 Hz, 2H, NCH$_2$C*H*$_2$C$_6$H$_5$), 4.46 (t, J = 7.3 Hz, 2H, NC*H*$_2$CH$_2$C$_6$H$_5$), 5.19 (s, 1H, H-3), 7.10–7.60 (m, 8H, H-7,8,9 + C_6H_5), 8.39 (m, J_o = 7.8 Hz, 1H, H-6), 11.43 (s, 1H, OH; disappeared with D_2O). ^{13}C NMR (75.5 MHz, DMSO-d_6, ppm): δ 33.26, 43.01, 80.63, 109.81, 114.88, 122.03, 125.05 (2C), 126.48, 128.31, 128.75, 130.55, 137.80, 148.17, 160.46, 169.56. Anal. $C_{18}H_{15}N_3O_2$ (C, H, N).

*6.1.2.7. 2-Hydroxy-10-[(methylthio)methyl]pyrimido[1,2-a]benzimidazol-4(10H)-one (**9g**).* Obtained (1.16 g, 89%) from **8g** (0.97 g); white crystals, m.p. 252–254 °C dec. (EtOH). IR (KBr, cm^{-1}): 3180–2195 br (zwitterionic form NH^+), 1675 sh and 1634 (CO), 1609, 1558 s, 1530 w, 1496. ^1H NMR (300 MHz, DMSO-d_6, ppm): δ 2.17 (s, 3H, NCH$_2$S*CH*$_3$), 5.20 (s, 1H, H-3), 5.44 (s, 2H, NC*H*$_2$SCH$_3$), 7.34–7.53 (m, 2H, H-7,8), 7.76 (m, J_o = 8 Hz, 1H, H-9), 8.42 (m, J_o = 7.8 Hz, 1H, H-6), 11.51 (broad s, 1H, OH; disappeared with D_2O). MS (ESI) m/z: monoisotopic expected MW = 261.06, experimental full scan m/z [M − H]$^-$ = 260.08. Fragment ions MS^2 [M − H]$^-$ 260.08: m/z 216.00 (100%), 200.08 (28%) ([M − H]$^-$ − CH$_2$=S=CH$_2$). Anal. $C_{12}H_{11}N_3O_2S$ (C, H, N, S).

*6.1.2.8. 2-Hydroxy-10-(2-pyridylmethyl)pyrimido[1,2-a]benzimidazol-4(10H)-one (**9h**).* Obtained (1.04 g, 71%) from **8h** (1.12 g); white crystals, m.p. 247–248 °C dec. (acetone). IR (KBr, cm^{-1}): 3200–2280 br (zwitterionic form NH^+), 1680 sh and 1634 (CO), 1610, 1558 s, 1530 sh, 1505. ^1H NMR (300 MHz, DMSO-d_6, ppm): δ 5.22 (s, 1H, H-3), 5.59 (s, 2H, NCH$_2$), 7.26–7.54 (m, 5H, H-7,8,9 + pyridyl H-3′,5′), 7.79 (m, 1H, pyridyl H-4′), 8.42–8.49 (m, 2H, H-6 + pyridyl H-6′), 11.47 (s, 1H, OH; disappeared with D_2O). ^{13}C NMR (75.5 MHz, DMSO-d_6, ppm): δ 47.25, 81.89, 111.04. 116.05, 122.55, 123.34, 123.88, 126.29, 126.35, 131.96, 138.13, 149.75, 150.31, 155.58, 161.52, 170.65. Anal. $C_{16}H_{12}N_4O_2$ (C, H, N).

*6.1.2.9. 2-Hydroxy-10-(3-pyridylmethyl)pyrimido[1,2-a]benzimidazol-4(10H)-one (**9i**).* Obtained (1.20 g, 82%) from **8i** (1.12 g); white crystals, m.p. 234–235 °C (acetone). IR (KBr, cm^{-1}): 3250–2300 br (zwitterionic form NH^+), 1674 br, s (CO), 1613, 1585, 1547 s, 1499. ^1H NMR (300 MHz, DMSO-d_6, ppm): δ 5.22 (s, 1H, H-3), 5.52 (s, 2H, NCH$_2$), 7.31–7.49 (m, 3H, H-7,8 + pyridyl H-5′), 7.65 (m, J_o = 7.8 Hz, 1H, H-9), 7.79 (m, 1H, pyridyl H-4′), 8.43 (m, J_o = 7.8 Hz, 1H, H-6), 8.51 (m, J_o = 4.8 Hz, J_m = 1.6 Hz, 1H, pyridyl H-6′), 8.74 (m, J_m = 1.6 Hz, 1H, pyridyl H-2′), 11.50 (broad s, 1H, OH; disappeared with D_2O). Anal. $C_{16}H_{12}N_4O_2$ (C, H, N).

*6.1.2.10. 2-Hydroxy-10-(4-pyridylmethyl)pyrimido[1,2-a]benzimidazol-4(10H)-one (**9j**).* Obtained (1.38 g, 94%) from **8j** (1.12 g); white crystals, m.p. 271–273 °C (EtOH). IR (KBr, cm^{-1}): 3089–2133 br (zwitterionic form NH^+), 1677 s (CO), 1610, 1591 w, 1568 w, 1546. ^1H NMR (200 MHz, DMSO-d_6, ppm): δ 5.22 (s, 1H, H-3), 5.53 (s, 2H, NCH$_2$), 7.23–7.61 (m, 5H, H-7,8,9 + pyridyl H-3′,5′), 8.39–8.62 (m, 3H, H-6 + pyridyl H-2′,6′), 11.50 (broad s, 1H, OH; disappeared with D_2O). MS (ESI) m/z: monoisotopic expected MW = 292.10 experimental full scan m/z [M + H]$^+$ = 293.17. Fragment ions MS^2 [M + H]$^+$ 293.17: m/z 251.08 (24%) ([M + H]$^+$ − CH$_2$=C=O), 223.17 (59%) ([M + H]$^+$ − CH$_2$=C=O, − C=O), 93.08 (100%) (•CH$_2$–  –N$^+$–H). Anal. $C_{16}H_{12}N_4O_2$ (C, H, N).

*6.1.2.11. 2-Hydroxy-10-[(4-methoxy-3,5-dimethyl-2-pyridyl)methyl]pyrimido[1,2-a]benzimidazol-4(10H)-one (**9k**).* Obtained (1.07 g, 61%) from **8k** (1.41 g); white crystals, m.p. 232–234 °C dec. (acetone/petroleum ether). IR (KBr, cm^{-1}): 3200–2330 br (zwitterionic form NH^+), 1679 s (CO), 1610, 1586. ^1H NMR (200 MHz, DMSO-d_6, ppm): δ 2.14 and 2.36 (2 s, 3H + 3H, 2 CH_3), 3.76 (s, 3H, OCH_3), 5.18 (s, 1H, H-3), 5.54 (s, 2H, NCH$_2$), 7.23–7.57 (m, 3H, H-7,8,9), 7.96 (s, 1H, pyridyl H-6′), 8.44 (m, J_o = 7.8 Hz, 1H, H-6), 10.40–11.80 (broad signal, 1H, OH; disappeared with D_2O). Anal. $C_{19}H_{18}N_4O_3$ (C, H, N).

*6.1.2.12. 10-[2-(Dimethylamino)ethyl]-2-hydroxypyrimido[1,2-a]benzimidazol-4(10H)-one (**9l**).* Obtained as perchlorate hemihydrate (**9l**·$HClO_4$·0.5H_2O) (1.11 g, 58%) from **8l** (1.02 g); white crystals, m.p. 260 °C dec. (EtOH). IR (KBr, cm^{-1}): 3470–2490 br (NH^+ + OH + crystallization H_2O), 1674 s (CO), 1612, 1566 s, 1490. ^1H NMR (300 MHz, DMSO-d_6, ppm): δ 2.96 [s, 6H, $NH^+(CH_3)_2$], 3.60 [m, 2H, NCH$_2$C*H*$_2$NH$^+$(CH$_3$)$_2$], 4.61 [m, 2H, NC*H*$_2$CH$_2$NH$^+$(CH$_3$)$_2$], 5.22 (s, 1H, H-3), 7.32–7.55 (m, 2H, H-7,8), 7.75 (m, J_o = 7.8 Hz, 1H, H-9), 8.42 (m, J_o = 7.8 Hz, 1H, H-6), 9.13 (broad s, 1H, NH^+; disappeared with D_2O), 11.41 (broad s, 1H, OH; disappeared with D_2O). MS (ESI) m/z: monoisotopic expected MW = 272.13, experimental MS full scan m/z [M + H]$^+$ = 273.17. Fragment ions MS^2 [M + H]$^+$ 273.17: m/z 228.08 (100%) ([M + H]$^+$ − HNMe$_2$). Anal. $C_{14}H_{16}N_4O_2$·$HClO_4$·0.5H_2O (C, H, N).

*6.1.2.13. 10-[2-(Diethylamino)ethyl]-2-hydroxypyrimido[1,2-a]benzimidazol-4(10H)-one (**9m**).* Obtained as perchlorate hemihydrate (**9m**···$HClO_4$···0.5H_2O) (1.41 g, 69%) from **8m** (1.16 g); white crystals, m.p. 266–267 °C dec. (EtOH). IR (KBr, cm^{-1}): 3470–2510 br (NH^+ + OH + crystallization H_2O), 1667 br, s (CO), 1615, 1562 br, s, 1493. ^1H NMR (300 MHz, DMSO-d_6, ppm): δ 1.18 [t, J = 7 Hz, 6H, $NH^+(CH_2CH_3)_2$], 3.33 [m, 4H, $NH^+(CH_2CH_3)_2$], 3.61 [m, 2H, NCH$_2$C*H*$_2$NH$^+$(CH$_2$CH$_3$)$_2$], 4.60 [m, 2H, NC*H*$_2$CH$_2$NH$^+$(CH$_2$CH$_3$)$_2$], 5.22 (s, 1H, H-3), 7.32–7.57 (m, 2H, H-7,8), 7.78 (m, J_o = 7.8 Hz, 1H,

H-9), 8.43 (m, J_o = 7.8 Hz, 1H, H-6), 8.88 (broad s, 1H, NH^+; disappeared with D_2O), 11.42 (broad s, 1H, OH; disappeared with D_2O). Anal. $C_{16}H_{20}N_4O_2 \cdot HClO_4 \cdot 0.5H_2O$ (C, H, N).

*6.1.2.14. 2-Hydroxy-10-[2-(1-pyrrolidinyl)ethyl]pyrimido[1,2-a]benzimidazol-4(10H)-one (**9n**).* Obtained as perchlorate (**9n**···$HClO_4$) (1.64 g, 82%) from **8n** (1.15 g); white crystals, m.p. 266–267 °C dec. (EtOH). IR (KBr, cm^{-1}): 3545–2510 br (NH^+ + OH), 1669 s (CO), 1614, 1562 s, 1492 w. ^1H NMR (300 MHz, DMSO-d_6, ppm): δ 1.70–2.20 (m, 4H, pyrrolidinyl β-CH_2's), 3.10–3.40 and 3.60–3.90 (2 m, 2H + 2H, pyrrolidinyl NH^+–CH_2's), 3.76 [m, 2H, $NCH_2CH_2NH^+$–pyrrolidinyl], 4.60 [m, 2H, $NCH_2CH_2NH^+$–pyrrolidinyl], 5.22 (s, 1H, H-3), 7.35–7.56 (m, 2H, H-7,8), 7.76 (m, J_o = 7.8 Hz, 1H, H-9), 8.44 (m, J_o = 7.8 Hz, 1H, H-6), 9.17 (broad s, 1H, NH^+; disappeared with D_2O), 11.39 (broad s, 1H, OH; disappeared with D_2O). Anal. $C_{16}H_{18}N_4O_2 \cdots HClO_4$ (C, H, N).

*6.1.2.15. 2-Hydroxy-10-[2-(4-morpholinyl)ethyl]pyrimido[1,2-a]benzimidazol-4(10H)-one (**9o**).* Obtained as perchlorate (**9o**···$HClO_4$) (1.64 g, 79%) from **8o** (1.23 g); white crystals, m.p. 276 °C dec. (EtOH). IR (KBr, cm^{-1}): 3450–2500 br (NH^+ + OH), 1678 sh and 1634 (CO), 1612, 1566 s, 1528 w, 1504. ^1H NMR (300 MHz, DMSO-d_6, ppm): δ 3.10–3.32 and 3.43–3.60 (2 m, 2H + 2H, NH^+–morpholinyl CH_2's), 3.64 [m, 2H, $NCH_2CH_2NH^+$–morpholinyl], 3.82–4.12 (m, 4H, morpholinyl O–CH_2's), 4.64 [m, 2H, $NCH_2CH_2NH^+$–morpholinyl], 5.21 (s, 1H, H-3), 7.35–7.57 (m, 2H, H-7,8), 7.78 (m, J_o = 7.8 Hz, 1H, H-9), 8.44 (m, J_o = 7.8 Hz, 1H, H-6), 9.17 (broad s, 1H, NH^+; disappeared with D_2O), 11.39 (broad s, 1H, OH; disappeared with D_2O). ^{13}C NMR (75.5 MHz, DMSO-d_6, ppm): δ 35.93, 51.49, 54.18, 63.26, 80.84, 110.03, 114.96, 122.60, 125.30, 125.63, 130.10, 149.06, 160.53, 169.26. Anal. $C_{16}H_{18}N_4O_3 \cdots HClO_4$ (C, H, N).

*6.1.3. General procedure for the synthesis of 10-substituted 2-chloropyrimido[1,2-a]benzimidazol-4(10H)-ones **10a–o***

A mixture of the proper 2-hydroxyderivative **9** (4.0 mmol) and an excess of $POCl_3$ (15.0 mL) was stirred at 130 °C (under reflux) for 2 h. The excess $POCl_3$ was removed in vacuo, and the thick and dark residue was treated with 5% $NaHCO_3$ (100 mL) and CH_2Cl_2 (100 mL) until it was completely dissolved. The mixture was stirred at room temperature for 30 min, treated with charcoal and filtered. The organic phase was then collected and the aqueous one was exhaustively extracted with CH_2Cl_2. The combined extracts, dried over anhydrous Na_2SO_4 were concentrated in vacuo and chromatographed on a silica gel column [eluants: CH_2Cl_2 for compounds **10a–g**, CH_2Cl_2–EtOAc (1:1) for compounds **10h–k**, acetone–EtOAc (1:1) for compounds **10l–o**]. The eluate collected, after removal of solvents, afforded the desired 2-chloroderivative **10** as a solid which was taken up in a small amount of petroleum ether, recovered by filtration, then crystallized from the proper solvent. According to this procedure the following compounds **10** were obtained.

*6.1.3.1. 2-Chloro-10-ethylpyrimido[1,2-a]benzimidazol-4(10H)-one (**10a**).* Obtained (0.88 g, 89%) from **9a** (0.92 g); white crystals, m.p. 199–201 °C (EtOAc/petroleum ether). IR (KBr, cm^{-1}): 1686 s (CO), 1605 sh, 1583 s, 1526. ^1H NMR (200 MHz, $CDCl_3$, ppm): δ 1.48 (t, J = 7 Hz, 3H, NCH_2CH_3), 4.36 (q, J = 7 Hz, 2H, NCH_2CH_3), 6.21 (s, 1H, H-3), 7.30–7.60 (m, 3H, H-7,8,9), 8.65 (m, J_o = 7.8 Hz, 1H, H-6). Anal. $C_{12}H_{10}ClN_3O$ (C, H, N).

*6.1.3.2. 2-Chloro-10-propylpyrimido[1,2-a]benzimidazol-4(10H)-one (**10b**).* Obtained (0.85 g, 81%) from **9b** (0.97 g); white crystals, m.p. 162–163 °C (EtOAc/petroleum ether). IR (KBr, cm^{-1}): 1673 s (CO), 1611, 1575 s, 1523. ^1H NMR (300 MHz, $CDCl_3$, ppm): δ 1.02 (t, J = 7 Hz, 3H, $NCH_2CH_2CH_3$), 1.95 (m, 2H, $NCH_2CH_2CH_3$), 4.26 (t, J = 7 Hz, 2H, $NCH_2CH_2CH_3$), 6.21 (s, 1H, H-3), 7.36–7.55 (m, 3H, H-7,8,9), 8.65 (m, J_o = 7.8 Hz, 1H, H-6). Anal. $C_{13}H_{12}ClN_3O$ (C, H, N).

*6.1.3.3. 2-Chloro-10-isopropylpyrimido[1,2-a]benzimidazol-4(10H)-one (**10c**).* Obtained (0.97 g, 93%) from **9c** (0.97 g); white crystals m.p. 202–203 °C (EtOAc). IR (KBr, cm^{-1}): 1687 (CO), 1615 w, 1605 w, 1578 s, 1527. ^1H NMR (200 MHz, $CDCl_3$, ppm): δ 1.72 [d, J = 7 Hz, 6H, $NCH(CH_3)_2$], 5.30 [m, 1H, $NCH(CH_3)_2$], 6.22 (s, 1H, H-3), 7.32–7.47 (m, 3H, H-7,8,9), 8.72 (m, J_o = 7.8 Hz, 1H, H-6). Anal. $C_{13}H_{12}ClN_3O$ (C, H, N).

*6.1.3.4. 10-Benzyl-2-chloropyrimido[1,2-a]benzimidazol-4(10H)-one (**10d**).* Obtained (0.76 g, 61%) from **9d** (1.17 g); white crystals, m.p. 256–257 °C (EtOAc). IR (KBr, cm^{-1}): 1674 s (CO), 1614 w, 1576 s, 1523. ^1H NMR (300 MHz, $CDCl_3$, ppm): δ 5.48 (s, 2H, NCH_2), 6.25 (s, 1H, H-3), 7.27–7.47 (m, 8H, H-7,8,9 + C_6H_5), 8.64 (m, J_o = 7.8 Hz, 1H, H-6). Anal. $C_{17}H_{12}ClN_3O$ (C, H, N).

*6.1.3.5. 2-Chloro-10-(1-phenylethyl)pyrimido[1,2-a]benzimidazol-4(10H)-one (**10e**).* Obtained (1.00 g, 77%) from **9e** (1.22 g); white crystals, m.p. 164–164.5 °C (EtOAc/petroleum ether). IR (KBr, cm^{-1}): 1681 s (CO), 1608 w, 1575 s, 1524. ^1H NMR (200 MHz, $CDCl_3$, ppm): δ 2.04 [d, J = 7 Hz, 3H, $NCH(CH_3)C_6H_5$], 6.29 (s, 1H, H-3), 6.45 [q, J = 7 Hz, 1H, $NCH(CH_3)C_6H_5$], 6.98–7.08 and 7.26–7.52 (2 m, 1H + 7H, H-7,8,9 + C_6H_5), 8.71 (m, J_o = 7.8 Hz, 1H, H-6). Anal. $C_{18}H_{14}ClN_3O$ (C, H, N).

*6.1.3.6. 2-Chloro-10-(2-phenylethyl)pyrimido[1,2-a]benzimidazol-4(10H)-one (**10f**).* Obtained (0.59 g, 45%) from **9f** (1.22 g); white crystals, m.p. 176–177 °C (EtOAc/petroleum ether). IR (KBr, cm^{-1}): 1682 s (CO), 1614 w, 1580 s, 1519. ^1H NMR (300 MHz, $CDCl_3$, ppm): δ 3.16 (t, J = 7.2 Hz, 2H, $NCH_2CH_2C_6H_5$), 4.50 (t, J = 7.2 Hz, 2H, $NCH_2CH_2C_6H_5$), 6.18 (s, 1H, H-3), 7.03–7.43 (m, 8H, H-7,8,9 + C_6H_5), 8.59 (m, J_o = 7.8 Hz, 1H, H-6). ^{13}C NMR (75.5 MHz, $CDCl_3$, ppm): δ 34.40, 44.14, 101.31, 109.04, 116.86, 123.24, 125.12, 126.51, 127.10, 128.77 (2C), 130.53, 137.11, 147.33, 158.00, 159.13. Anal. $C_{18}H_{14}ClN_3O$ (C, H, N).

*6.1.3.7. 2-Chloro-10-[(methylthio)methyl]pyrimido[1,2-a]benzimidazol-4(10H)-one (**10g**).* Obtained (0.51 g, 45%) from **8g** (1.05 g); white crystals, m.p. 256–257 °C (EtOAc). IR (KBr, cm^{-1}): 1680 s (CO), 1605 w, 1581 s, 1524. ^1H NMR (200 MHz, $CDCl_3$, ppm): δ 2.24 (s, 3H, NCH_2SCH_3), 5.41 (s, 2H, NCH_2SCH_3), 6.28 (s, 1H, H-3), 7.39–7.64 (m, 3H, H-7,8,9), 8.67 (m, J_o = 7.8 Hz, 1H, H-6). MS (ESI) m/z: monoisotopic expected MW = 279.02; experimental full scan m/z [M − H]$^-$ = 278.00. Fragment ions MS^2 [M − H]$^-$ 278.00: m/z 242.00 (100%) ([M − H]$^-$ − HCl), 231.00 (81%). Anal. $C_{12}H_{10}ClN_3OS$ (C, H, N, S).

*6.1.3.8. 2-Chloro-10-(2-pyridylmethyl)pyrimido[1,2-a]benzimidazol-4(10H)-one (**10h**).* Obtained (0.78 g, 63%) from **9h** (1.17 g); white crystals, m.p. 194–195 °C (EtOAc). IR (KBr, cm^{-1}): 1686 s (CO), 1613 w, 1583 s, 1525. ^1H NMR (300 MHz, $CDCl_3$, ppm): δ 5.60 (s, 2H, NCH_2), 6.24 (s, 1H, H-3), 7.21–7.47 (m, 5H, H-7,8,9 + pyridyl H-3′,5′), 7.67 (m, 1H, pyridyl H-4′), 8.49–8.63 (m, 2H, H-6 + pyridyl H-6′). ^{13}C NMR (75.5 MHz, $CDCl_3$, ppm): δ 47.85, 101.70, 110.25, 116.79, 122.18, 123.32, 123.56, 125.32, 126.73, 130.64, 137.23, 147.73, 149.75, 154.03, 158.04, 159.12. Anal. $C_{16}H_{11}ClN_4O$ (C, H, N).

*6.1.3.9. 2-Chloro-10-(3-pyridylmethyl)pyrimido[1,2-a]benzimidazol-4(10H)-one (**10i**).* Obtained (0.65 g, 52%) from **9i** (1.17 g); white crystals, m.p. 243–244 °C (EtOAc). IR (KBr, cm^{-1}): 1672 s (CO), 1613 w, 1605 w, 1579 s, 1571 s, 1523. ^1H NMR (200 MHz, $CDCl_3$, ppm): δ 5.53 (s, 2H, NCH_2), 6.30 (s, 1H, H-3), 7.25–7.80 (m, 5H, H-7,8,9 + pyridyl H-4′,5′), 8.55–8.91 (m, 3H, H-6 + pyridyl H-2′,6′). Anal. $C_{16}H_{11}ClN_4O$ (C, H, N).

*6.1.3.10. 2-Chloro-10-(4-pyridylmethyl)pyrimido[1,2-a]benzimidazol-4(10H)-one (**10j**).* Obtained (0.53 g, 44%) from **9j** (1.17 g); white crystals, m.p. 265–267 °C dec. (EtOAc). IR (KBr, cm^{-1}): 1671 s (CO), 1582 s, 1526. ^1H NMR (200 MHz, $CDCl_3$, ppm): δ 5.52 (s, 2H, NCH_2), 6.31 (s, 1H, H-3), 7.15–7.30 and 7.38–7.57 (2 m, 3H + 2H, H-7,8,9 + pyridyl H-3′,5′), 8.55–8.75 (m, 3H, H-6 + pyridyl H-2′,6′). MS (ESI) *m/z*: monoisotopic expected MW = 310.06, experimental full scan *m/z* $[M + H]^+$ = 311.17. Fragment ions MS^2 $[M + H]^+$ 311.17: *m/z* 275.17 (100%) ($[M + H]^+ - HCl$), 247.17 (15%) ($[M + H]^+ - HCl, - C{=}O$), 93.08 (26%) (•CH_2–  –H). Anal. $C_{16}H_{11}ClN_4O$ (C, H, N).

*6.1.3.11. 2-Chloro-10-[(4-methoxy-3,5-dimethyl-2-pyridyl)methyl]pyrimido[1,2-a]benzimidazol-4(10H)-one (**10k**).* Obtained (0.87 g, 59%) from **9k** (1.40 g); white crystals, m.p. 214–215 °C (EtOAc/petroleum ether). IR (KBr, cm^{-1}): 1683 s (CO), 1612 w, 1580 s, 1529. ^1H NMR (200 MHz, $CDCl_3$, ppm): δ 2.30 and 2.36 (2 s, 3H + 3H, 2 CH_3), 3.83 (s, 3H, OCH_3), 5.69 (s, 2H, NCH_2), 6.27 (s, 1H, H-3), 7.38–7.58 (m, 3H, H-7,8,9), 8.20 (s, 1H, pyridyl H-6′), 8.68 (m, J_o = 7.8 Hz, 1H, H-6). Anal. $C_{19}H_{17}ClN_4O_2$ (C, H, N).

*6.1.3.12. 2-Chloro-10-[2-(dimethylamino)ethyl]pyrimido[1,2-a]benzimidazol-4(10H)-one (**10l**).* Obtained (0.44 g, 38%) from **9l**·$HClO_4$·0.5H_2O (1.53 g); white crystals, m.p. 159–160 °C (diisopropyl ether). IR (KBr, cm^{-1}): 1679 s (CO), 1615 w, 1578 s, 1521. ^1H NMR (200 MHz, $CDCl_3$, ppm): δ 2.35 [s, 6H, $N(CH_3)_2$], 2.81 [t, *J* = 7 Hz, 2H, $NCH_2CH_2N(CH_3)_2$], 4.42 [t, *J* = 7 Hz, 2H, $NCH_2CH_2N(CH_3)_2$], 6.24 (s, 1H, H-3), 7.35–7.63 (m, 3H, H-7,8,9), 8.68 (m, J_o = 7.8 Hz, 1H, H-6). MS (ESI) *m/z*: monoisotopic expected MW = 290.09, experimental full scan *m/z* $[M + H]^+$ = 291.17. Fragment ions MS^2 $[M + H]^+$ 291.17: *m/z* 228.08 (10%), 210.08 (100%) ($[M + H]^+ - HNMe_2, - HCl$), 182.17 (72%) ($[M + H]^+ - HNMe_2, - HCl, - C{=}O$), 155.08 (21%). Anal. $C_{14}H_{15}ClN_4O$ (C, H, N).

*6.1.3.13. 2-Chloro-10-[2-(diethylamino)ethyl]pyrimido[1,2-a]benzimidazol-4(10H)-one (**10m**).* Obtained (0.45 g, 35%) from **9m**·$HClO_4$·0.5H_2O (1.64 g); white crystals, m.p. 125–127 °C (diisopropyl ether). IR (KBr, cm^{-1}): 1680 s (CO), 1614 w, 1605 w, 1587 s, 1524. ^1H NMR (300 MHz, $CDCl_3$, ppm): δ 0.90 [t, *J* = 7 Hz, 6H, $N(CH_2CH_3)_2$], 2.55 [q, *J* = 7 Hz, 4H, $N(CH_2CH_3)_2$], 2.87 [t, *J* = 7 Hz, 2H, $NCH_2CH_2N(CH_2CH_3)_2$], 4.34 [t, *J* = 7 Hz, 2H, $NCH_2CH_2N(CH_2CH_3)_2$], 6.19 (s, 1H, H-3), 7.33–7.55 (m, 3H, H-7,8,9), 8.63 (m, J_o = 7.8 Hz, 1H, H-6). Anal. $C_{16}H_{19}ClN_4O$ (C, H, N).

*6.1.3.14. 2-Chloro-10-[2-(1-pyrrolidinyl)ethyl]pyrimido[1,2-a]benzimidazol-4(10H)-one (**10n**).* Obtained (0.70 g, 55%) from **9n**···$HClO_4$ (1.60 g); white crystals, m.p. 142–143 °C (EtOAc/petroleum ether). IR (KBr, cm^{-1}): 1682 s (CO), 1589 s, 1526. ^1H NMR (200 MHz, $CDCl_3$, ppm): δ 1.82 (m, 4H, pyrrolidinyl β-CH_2's), 2.66 (m, 4H, pyrrolidinyl N–CH_2's), 2.98 [t, *J* = 7 Hz, 2H, NCH_2CH_2N–pyrrolidinyl], 4.44 [t, *J* = 7 Hz, 2H, NCH_2CH_2N–pyrrolidinyl], 6.23 (s, 1H, H-3), 7.34–7.62 (m, 3H, H-7,8,9), 8.67 (m, J_o = 7.8 Hz, 1H, H-6). Anal. $C_{16}H_{17}ClN_4O$ (C, H, N).

*6.1.3.15. 2-Chloro-10-[2-(4-morpholinyl)ethyl]pyrimido[1,2-a]benzimidazol-4(10H)-one (**10o**).* Obtained (0.69 g, 52%) from **9o**···$HClO_4$ (1.66 g); white crystals, m.p. 189–191 °C (EtOAc). IR (KBr, cm^{-1}): 1682 s (CO), 1586 s, 1527. ^1H NMR (300 MHz, $CDCl_3$, ppm): δ 2.54 (t, *J* = 4.6 Hz, 4H, morpholinyl N–CH_2's), 2.80 [t, *J* = 6.3 Hz, 2H, NCH_2CH_2N–morpholinyl], 3.60 (t, *J* = 4.6 Hz, 4H, morpholinyl O–CH_2's), 4.40 [t, *J* = 6.3 Hz, 2H, NCH_2CH_2N–morpholinyl], 6.19 (s, 1H, H-3), 7.31–7.58 (m, 3H, H-7,8,9), 8.62 (m, J_o = 7.8 Hz, 1H, H-6). ^{13}C NMR (75.5 MHz, $CDCl_3$, ppm): δ 39.94, 53.70, 55.96, 66.83, 101.19, 109.19, 116.97, 123.32, 125.36, 126.54, 130.63, 147.79, 157.94, 159.17. Anal. $C_{16}H_{17}ClN_4O_2$ (C, H, N).

*6.1.4. General procedure for the synthesis of 10-substituted 2-(1-piperazinyl)pyrimido[1,2-a]benzimidazol-4(10H)-ones **6a–o***

A mixture of 2.0 mmol of the proper chloroderivative **10**, piperazine (1.72 g, 20.0 mmol) and ethanol (60 mL) was heated at reflux for 3 h. The solution obtained was poured into water (250 mL): only in the case of reaction with the 10-benzylderivative **10d**, pure compound **6d** separated out as a white solid which was collected by filtration, dried and crystallized from proper solvent. In all other cases an emulsion was obtained which was exhaustively extracted with CH_2Cl_2. The combined extracts, after drying and removal of solvents, afforded an oily or nearly solid residue which, in most cases, was treated with a small amount of EtOAc and diethyl ether to give pure compound **6** as a whitish solid which was then crystallized from the suitable solvent (compounds **6a**,**f**,**g**–**l**,**n**,**o**). Compounds **6b**,**c** required a preliminary purification by chromatography on a silica gel column, eluting with THF to remove some impurities, then with MeOH to recover pure compound **6**. In the case of compounds **6e**,**m** the final oily residue was treated with a solution of maleic acid (0.29 g, 2.5 mmol) in anhydrous ethanol to give the corresponding pure maleates (**6e**···$C_4H_4O_4$ or **6m**···$2C_4H_4O_4$) as a white solid which was then crystallized from anhydrous ethanol.

According to procedures above described, the following compounds were prepared.

*6.1.4.1. 10-Ethyl-2-(1-piperazinyl)pyrimido[1,2-a]benzimidazol-4(10H)-one (**6a**).* Obtained (0.44 g, 74%) from **10a** (0.50 g); white crystals, m.p. 212–214 °C (EtOAc). IR (KBr, cm^{-1}): 3316 w and 3287 w (NH), 1661 s (CO), 1609, 1585 s, 1528. ^1H NMR (200 MHz, $CDCl_3$, ppm): δ 1.42 (t, *J* = 7 Hz, 3H, NCH_2CH_3), 2.39 (s, 1H, NH; disappeared with D_2O), 2.97 [t, *J* = 5 Hz, 4H, piperazinyl $(CH_2)_2NH$], 3.64 [t, *J* = 5 Hz, 4H, piperazinyl $(CH_2)_2N$–], 4.21 (q, *J* = 7 Hz, 2H, NCH_2CH_3), 5.30 (s, 1H, H-3), 7.19–7.41 (m, 3H, H-7,8,9), 8.55 (m, J_o = 7.8 Hz, 1H, H-6). Anal. $C_{16}H_{19}N_5O$ (C, H, N).

*6.1.4.2. 2-(1-Piperazinyl)-10-propylpyrimido[1,2-a]benzimidazol-4(10H)-one (**6b**).* Obtained (0.35 g, 56%) from **10b** (0.52 g); white crystals, m.p. 188–189 °C (EtOAc/diethyl ether). IR (KBr, cm^{-1}): 3319 w and 3288 w (NH), 1668 s (CO), 1610, 1584, 1529. ^1H NMR (200 MHz, $CDCl_3$, ppm): δ 1.01 (t, *J* = 7 Hz, 3H, $NCH_2CH_2CH_3$), 1.75–2.15 (m, 3H, $NCH_2CH_2CH_3$ + NH; 2H after treatment with D_2O), 2.99 [t, *J* = 5 Hz, 4H, piperazinyl $(CH_2)_2NH$], 3.64 [t, *J* = 5 Hz, 4H, piperazinyl $(CH_2)_2N$–], 4.16 (t, *J* = 7 Hz, 2H, $NCH_2CH_2CH_3$), 5.32 (s, 1H, H-3), 7.15–7.48 (m, 3H, H-7,8,9), 8.58 (m, J_o = 7.8 Hz, 1H, H-6). Anal. $C_{17}H_{21}N_5O$ (C, H, N).

*6.1.4.3. 10-Isopropyl-2-(1-piperazinyl)pyrimido[1,2-a]benzimidazol-4(10H)-one (**6c**).* Obtained as monohydrate (**6c**·H_2O) (0.46 g, 70%) from **10c** (0.52 g); white crystals m.p. 152–153 °C (EtOAc). IR (KBr, cm^{-1}): 3465 (crystallization H_2O), 3232 (NH), 1659 s (CO), 1608, 1571, 1531. ^1H NMR (200 MHz, $CDCl_3$, ppm): δ 1.64 [d, *J* = 7 Hz, 6H, $NCH(CH_3)_2$], 2.19 (s, 3H, NH + crystallization H_2O; disappeared with D_2O), 2.99 [t, *J* = 5 Hz, 4H, piperazinyl $(CH_2)_2NH$], 3.64 [t, *J* = 5 Hz, 4H, piperazinyl $(CH_2)_2N$–], 5.04 [m, 1H, $NCH(CH_3)_2$], 5.31 (s, 1H, H-3), 7.16–7.43 (m, 3H, H-7,8,9), 8.59 (m, J_o = 7.8 Hz, 1H, H-6). Anal. $C_{17}H_{21}N_5O·H_2O$ (C, H, N).

*6.1.4.4. 10-Benzyl-2-(1-piperazinyl)pyrimido[1,2-a]benzimidazol-4(10H)-one (**6d**).* Obtained (0.53 g, 74%) from **10d** (0.62 g); white crystals, m.p. 165–166 °C (EtOAc/petroleum ether). IR (KBr, cm^{-1}): 3215 (NH), 1667 s (CO), 1610, 1583, 1532. ^1H NMR (300 MHz, $CDCl_3$, ppm): δ 1.89 (s, 1H, NH; disappeared with D_2O), 2.96 [t, *J* = 5 Hz, 4H, piperazinyl $(CH_2)_2NH$], 3.64 [t, *J* = 5 Hz, 4H, piperazinyl $(CH_2)_2N$–], 5.33 (s, 1H, H-3), 5.36 (s, 2H, NCH_2), 7.11–7.36 (m, 8H, H-7,8,9 + C_6H_5), 8.56 (m, J_o = 7.8 Hz, 1H, H-6). Anal. $C_{21}H_{21}N_5O$ (C, H, N).

*6.1.4.5. 10-(1-Phenylethyl)-2-(1-piperazinyl)pyrimido[1,2-a]benzimidazol-4(10H)-one (**6e**).* Obtained as maleate (**6e**···$C_4H_4O_4$) (0.68 g, 70%) from **10e** (0.65 g); white crystals, m.p. 146–148 °C (anhydrous EtOH/Et_2O). The ^{1}H NMR and IR spectra were recorded on the free amine obtained (as amorphous solid, m.p. 110–112 °C) from the analytical sample of maleate, by treatment with aqueous $NaHCO_3$, exhaustive extraction with CH_2Cl_2 and removal of solvent. IR (KBr, cm^{-1}): 3220 (NH), 1663 s (CO), 1610, 1579, 1533. ^{1}H NMR (200 MHz, $CDCl_3$, ppm): δ 2.00 [d, J = 7 Hz, 3H, NCH(CH_3)C_6H_5], 2.67 (s, 1H, NH; disappeared with D_2O), 3.19 [t, J = 5 Hz, 4H, piperazinyl (CH_2)$_2$NH], 3.88 [t, J = 5 Hz, 4H, piperazinyl (CH_2)$_2$N–], 5.42 (s, 1H, H-3), 6.27 [q, J = 7 Hz, 1H, NCH(CH_3)C_6H_5], 6.91–7.03 and 7.15–7.44 (m, 1H + 7H, H-7,8,9 + C_6H_5), 8.61 (m, J_o = 7.8 Hz, 1H, H-6). Anal. $C_{22}H_{23}N_5O$···$C_4H_4O_4$ (C, H, N).

*6.1.4.6. 10-(2-Phenylethyl)-2-(1-piperazinyl)pyrimido[1,2-a]benzimidazol-4(10H)-one (**6f**).* Obtained (0.63 g, 84%) from **10f** (0.65 g); white crystals, m.p. 137–138 °C (EtOAc/diisopropyl ether). IR (KBr, cm^{-1}): 3237 (NH), 1675 s (CO), 1612, 1584 s, 1530. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 2.37 (s, 1H, NH; disappeared with D_2O), 2.97 [t, J = 5 Hz, 4H, piperazinyl (CH_2)$_2$NH], 3.11 (t, J = 7.3 Hz, 2H, $NCH_2CH_2C_6H_5$), 3.59 [t, J = 5 Hz, 4H, piperazinyl (CH_2)$_2$N–], 4.36 (t, J = 7.3 Hz, 2H, $NCH_2CH_2C_6H_5$), 5.27 (s, 1H, H-3), 7.03–7.32 (m, 8H, H-7,8,9 + C_6H_5), 8.52 (m, J_o = 7.8 Hz, 1H, H-6). ^{13}C NMR (75.5 MHz, $CDCl_3$, ppm): δ 34.27, 43.57, 45.74, 45.81, 78.67, 108.10, 116.11, 122.24, 124.72, 126.01, 126.87, 128.70, 128.72, 130.66, 137.77, 148.23, 161.25, 162.47. Anal. $C_{22}H_{23}N_5O$ (C, H, N).

*6.1.4.7. 10-[(Methylthio)methyl]-2-(1-piperazinyl)pyrimido[1,2-a]benzimidazol-4(10H)-one (**6g**).* Obtained (0.34 g, 52%) from **10g** (0.56 g); white crystals, m.p. 175–177 °C (EtOAc/Et_2O). IR (KBr, cm^{-1}): 3330 (NH), 1668 s (CO), 1611, 1585, 1537. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 2.18 (s, 4H, NCH_2SCH_3 + NH; 3H after treatment with D_2O), 2.98 [t, J = 5 Hz, 4H, piperazinyl (CH_2)$_2$NH], 3.64 [t, J = 5 Hz, 4H, piperazinyl (CH_2)$_2$N–], 5.27 (s, 2H, NCH_2SCH_3), 5.31 (s, 1H, H-3), 7.28–7.43 (m, 3H, H-7,8,9), 8.55 (m, J_o = 7.8 Hz, 1H, H-6). MS (ESI) m/z: monoisotopic expected MW = 329.13, experimental full scan m/z [M + H]$^+$ = 330.17. Fragment ions MS^2 [M + H]$^+$: m/z 282.17 (100%) ([M + H]$^+$ − CH_3SH), 270.17 (52%), 239.17 (8%) ([M + H]$^+$ − CH_3SH, − CH_2=CH–NH_2). Anal. $C_{16}H_{19}N_5OS$ (C, H, N, S).

*6.1.4.8. 2-(1-Piperazinyl)-10-(2-pyridylmethyl)pyrimido[1,2-a]benzimidazol-4(10H)-one (**6h**).* Obtained (0.49 g, 68%) from **10h** (0.62 g); white crystals, m.p. 175–176 °C (EtOAc). IR (KBr, cm^{-1}): 3240 (NH), 1665 s (CO), 1612, 1584, 1532. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 2.17 (s, 1H, NH; disappeared with D_2O), 2.94 [t, J = 5 Hz, 4H, piperazinyl (CH_2)$_2$NH], 3.62 [t, J = 5 Hz, 4H, piperazinyl (CH_2)$_2$N–], 5.34 (s, 1H, H-3), 5.49 (s, 2H, NCH_2), 7.13–7.32 (m, 5H, H-7,8,9 + pyridyl H-3′,5′), 7.63 (m, 1H, pyridyl H-4′), 8.53–8.61 (m, 2H, H-6 + pyridyl H-6′). ^{13}C NMR (75.5 MHz, $CDCl_3$, ppm): δ 45.75, 45.87, 47.41, 78.87, 109.11, 116.08, 121.60, 122.66, 123.01, 124.96, 126.18, 130.73, 137.16, 148.58, 149.61, 155.19, 161.27, 162.51. Anal. $C_{20}H_{20}N_6O$ (C, H, N).

*6.1.4.9. 2-(1-Piperazinyl)-10-(3-pyridylmethyl)pyrimido[1,2-a]benzimidazol-4(10H)-one (**6i**).* Obtained as hemihydrate (**6i**·$0.5H_2O$) (0.45 g, 61%) from **10i** (0.62 g); white crystals, m.p. 185–186 °C (EtOAc). IR (KBr, cm^{-1}): 3391 (crystallization H_2O), 3316 (NH), 1675 s (CO), 1613, 1592, 1539. ^{1}H NMR (200 MHz, $CDCl_3$, ppm): δ 1.82 (s, 2H, NH + crystallization H_2O; disappeared with D_2O), 2.94 [t, J = 5 Hz, 4H, piperazinyl (CH_2)$_2$NH], 3.61 [t, J = 5 Hz, 4H, piperazinyl (CH_2)$_2$N–], 5.31 (s, 1H, H-3), 5.36 (s, 2H, NCH_2), 7.11–7.37 (m, 4H, H-7,8,9 + pyridyl H-5′), 7.63 (m, 1H, pyridyl H-4′), 8.50–8.62 (m, 2H, H-6 + pyridyl H-6′), 8.71 (s, 1H, pyridyl H-2′). Anal. $C_{20}H_{20}N_6O\cdot 0.5H_2O$ (C, H, N).

*6.1.4.10. 2-(1-Piperazinyl)-10-(4-pyridylmethyl)pyrimido[1,2-a]benzimidazol-4(10H)-one (**6j**).* Obtained as hemihydrate (**6j**·$0.5H_2O$) (0.60 g, 81%) from **10j** (0.62 g); white crystals, m.p. 202–204 °C (EtOAc). IR (KBr, cm^{-1}): 3422 (crystallization H_2O), 3320 (NH), 1669 s (CO), 1615, 1593, 1539. ^{1}H NMR (200 MHz, $CDCl_3$, ppm): δ 1.84 (s, 2H, NH + crystallization H_2O; disappeared with D_2O), 2.96 [t, J = 5 Hz, 4H, piperazinyl (CH_2)$_2$NH], 3.62 [t, J = 5 Hz, 4H, piperazinyl (CH_2)$_2$N–], 5.36 (s, 1H, H-3), 5.39 (s, 2H, NCH_2), 7.00–7.40 (m, 5H, H-7,8,9 + pyridyl H-3′,5′), 8.47–8.67 (m, 3H, H-6 + pyridyl H-2′,6′). MS (ESI) m/z: monoisotopic expected MW = 360.17, experimental full scan m/z [M + H]$^+$ = 361.25. Fragment ions MS^2 [M + H]$^+$ 361.25: m/z 318.17 (100%) ([M + H]$^+$ − CH_2=CH–NH_2), 226.08 (32%) ([M + H]$^+$ − CH_2=CH–NH_2, − •CH_2– ). Anal. $C_{20}H_{20}N_6O\cdot 0.5H_2O$ (C, H, N).

*6.1.4.11. 2-(1-Piperazinyl)-10-[(4-methoxy-3,5-dimethyl-2-pyridyl)methyl]pyrimido[1,2-a]benzimidazol-4(10H)-one (**6k**).* Obtained as hemihydrate (**6k**·$0.5H_2O$) (0.79 g, 92%) from **10k** (0.74 g); white crystals, m.p. 206–208 °C (EtOAc). IR (KBr, cm^{-1}): 3440 (crystallization H_2O), 3283 (NH), 1670 s (CO), 1612, 1590, 1540. ^{1}H NMR (200 MHz, $CDCl_3$, ppm): δ 2.27 and 2.29 (2 s, 8H, 2 CH_3 + NH + crystallization H_2O; 6H after treatment with D_2O), 3.13 [t, J = 5 Hz, 4H, piperazinyl (CH_2)$_2$NH], 3.75 (s, 3H, OCH_3), 3.82 [t, J = 5 Hz, 4H, piperazinyl (CH_2)$_2$N–], 5.39 (s, 1H, H-3), 5.49 (s, 2H, NCH_2), 7.20–7.42 (m, 3H, H-7,8,9), 8.22 (s, 1H, pyridyl H-6′), 8.56 (m, J_o = 7.8 Hz, 1H, H-6). Anal. $C_{23}H_{26}N_6O_2\cdot 0.5H_2O$ (C, H, N).

*6.1.4.12. 10-[2-(Dimethylamino)ethyl]-2-(1-piperazinyl)pyrimido[1,2-a]benzimidazol-4(10H)-one (**6l**).* Obtained (0.45 g, 66%) from **10l** (0.58 g); white crystals, m.p. 124–125 °C (EtOAc/petroleum ether). IR (KBr, cm^{-1}): 3311 (NH), 1672 s (CO), 1612, 1587, 1537. ^{1}H NMR (200 MHz, $CDCl_3$, ppm): δ 2.01 (s, 1H, NH; disappeared with D_2O), 2.36 [s, 6H, N(CH_3)$_2$], 2.76 [t, J = 7 Hz, 2H, $NCH_2CH_2N(CH_3)_2$], 2.98 [t, J = 5 Hz, 4H, piperazinyl (CH_2)$_2$NH], 3.64 [t, J = 5 Hz, 4H, piperazinyl (CH_2)$_2$N–], 4.30 [t, J = 7 Hz, 2H, $NCH_2CH_2N(CH_3)_2$], 5.33 (s, 1H, H-3), 7.22–7.47 (m, 3H, H-7,8,9), 8.57 (m, J_o = 7.8 Hz, 1H, H-6). MS (ESI) m/z: monoisotopic expected MW = 340.20, experimental full scan m/z [M + H]$^+$ = 341.25. Fragment ions MS^2 [M + H]$^+$ 341.25: m/z 298.17 (43%) ([M + H]$^+$ − CH_2=CH–NH_2), 296.00 (43%) ([M + H]$^+$ − $HNMe_2$), 270.33 (100%) ([M + H]$^+$− CH_2=CH–NH_2, − C=O), 155.08 (21%). Anal. $C_{18}H_{24}N_6O$ (C, H, N).

*6.1.4.13. 10-[2-(Diethylamino)ethyl]-2-(1-piperazinyl)pyrimido[1,2-a]benzimidazol-4(10H)-one (**6m**).* As dimaleate (**6m**···$2H_4C_4O_4$) (0.68 g, 57%) from **10m** (0.64 g); whitish crystals, m.p. 156–158 °C (anhydrous EtOH). The ^{1}H NMR and IR spectra were recorded on the free amine obtained (as a whitish resin) from the analytical sample of maleate as above described for compound **6e**. IR ($CHCl_3$, cm^{-1}): 1667 s (CO), 1613, 1590 s, 1520 (NH stretching was not observable). ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 0.97 [t, J = 7 Hz, 6H, N(CH_2CH_3)$_2$], 2.12 (s, 1H, NH; disappeared with D_2O), 2.59 [q, J = 7 Hz, 4H, N(CH_2CH_3)$_2$], 2.84 [t, J = 7 Hz, 2H, $NCH_2CH_2N(C_2H_5)_2$], 3.02 [t, J = 5 Hz, 4H, piperazinyl (CH_2)$_2$NH], 3.67 [t, J = 5 Hz, 4H, piperazinyl (CH_2)$_2$N–], 4.24 [t, J = 7 Hz, 2H, $NCH_2CH_2N(C_2H_5)_2$], 5.30 (s, 1H, H-3), 7.23–7.44 (m, 3H, H-7,8,9), 8.58 (m, J_o = 7.8 Hz, 1H, H-6). Anal. $C_{20}H_{28}N_6O$···2 $H_4C_4O_4$ (C, H, N).

*6.1.4.14. 2-(1-Piperazinyl)-10-[2-(1-pyrrolidinyl)ethyl]pyrimido[1,2-a]benzimidazol-4(10H)-one (**6n**).* Obtained (0.63 g, 86%) from **10n** (0.63 g); white crystals, m.p. 152–153 °C (EtOAc). IR (KBr, cm^{-1}): 3280 (NH), 1671 s (CO), 1610, 1585, 1537. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.80 (m, 4H, pyrrolidinyl β-CH_2's), 2.16 (s, 1H, NH; disappeared with D_2O), 2.63 (m, 4H, pyrrolidinyl N–CH_2's), 2.90 [t, J = 7 Hz, 2H, NCH_2CH_2N–pyrrolidinyl], 2.96 [t, J = 5 Hz, 4H, piperazinyl (CH_2)$_2$NH], 3.62 [t, J = 5 Hz, 4H, piperazinyl (CH_2)$_2$N–],

4.31 [t, J = 7 Hz, 2H, NCH_2CH_2N–pyrrolidinyl], 5.30 (s, 1H, H-3), 7.23–7.39 (m, 3H, H-7,8,9), 8.54 (m, J_o = 7.8 Hz, 1H, H-6). Anal. $C_{20}H_{26}N_6O$ (C, H, N).

*6.1.4.15. 10-[2-(4-Morpholinyl)ethyl]-2-(1-piperazinyl)pyrimido[1,2-a]benzimidazol-4(10H)-one (**6o**).* Obtained (0.59 g, 77%) from **10o** (0.66 g); white crystals, m.p. 172–173.5 °C (EtOAc). IR (KBr, cm^{-1}): 3265 (NH), 1686 s (CO), 1611, 1593 s, 1535. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 2.04 (s, 1H, NH; disappeared with D_2O), 2.55 (t, J = 4.6 Hz, 4H, morpholinyl N–CH_2's), 2.78 [t, J = 6.6 Hz, 2H, NCH_2CH_2N–morpholinyl], 2.95 [t, J = 5 Hz, 4H, piperazinyl (CH_2)$_2$NH], 3.53–3.70 [m, 8H, morpholinyl O–CH_2's + piperazinyl (CH_2)$_2$N–], 4.29 [t, J = 6.6 Hz, 2H, NCH_2CH_2N–morpholinyl], 5.30 (s, 1H, H-3), 7.23–7.40 (m, 3H, H-7,8,9), 8.55 (m, J_o = 7.8 Hz, 1H, H-6). ^{13}C NMR (75.5 MHz, $CDCl_3$, ppm): δ 39.31, 45.82, 45.87, 53.75, 55.86, 66.82, 78.71, 108.21, 116.24, 122.36, 124.75, 126.21, 130.79, 148.59, 161.26, 162.52. Anal. $C_{20}H_{26}N_6O_2$ (C, H, N).

*6.1.5. N,N-Diethyl-N′-2-(1-ethylbenzimidazolyl)propanediamide (**12**)*

PCl_5 (1.46 g, 7.0 mmol) was slowly added to an ice-cooled solution of *N,N*-diethylmalonamic acid **11** [7] (1.05 g, 6.6 mmol) and 1-ethyl-1*H*-benzimidazol-2-amine (**8a**) (0.97 g, 6.0 mmol). The mixture was stirred at room temperature for 3 h, then heated at reflux for 1 h and finally poured into cold water. After alkalinization with Na_2CO_3, the mixture was exhaustively extracted with CH_2Cl_2. The combined extracts, dried over Na_2SO_4, afforded an oily residue which was chromatographed on a silica gel column eluting with acetone/EtOAc (1:1). The eluate collected, after removal of solvent, gave a thick oil from which pure compound **12** separated out as a whitish solid, after treatment with a small amount of Et_2O/diisopropyl ether (1:1) (0.56 g, 31%); white crystals, m.p. 94–95 °C (Et_2O/diisopropyl ether). IR (KBr, cm^{-1}): 3268 (NH), 1628 s (CO), 1599 s (CO), 1560. ^{1}H NMR (200 MHz, $CDCl_3$, ppm): δ 1.19 and 1.26 [2t, J = 7 Hz, 3H + 3H, CON(CH_2CH_3)$_2$], 1.39 (t, J = 7 Hz, 3H, NCH$_2$CH_3), 3.44 and 3.46 [2q, J = 7 Hz, 2H + 2H, CON(CH_2CH$_3$)$_2$], 3.64 (s, 2H, COCH_2CO), 4.20 (q, J = 7 Hz, 2H, NCH_2CH$_3$), 7.18–7.42 (m, 4H, H-4,5,6,7), 9.20–10.70 (broad signal, 1H, NH; disappeared with D_2O). MS (ESI) m/z: monoisotopic expected MW = 302.17, experimental full scan m/z [M + H]$^+$ = 303.17. Fragment ions MS^2 [M + H]$^+$ 303.17: m/z 230.17 (51%) ([M + H]$^+$ − HNEt$_2$), 188.17 (26%) ([M + H]$^+$ − CH$_3$CONEt$_2$), 162.17 (100%) ([M + H]$^+$ − O=C=CH–CONEt$_2$). Anal. $C_{16}H_{22}N_4O_2$ (C, H, N).

*6.1.6. Cyclization of compound **12** to 10-ethyl-4-(diethylamino) pyrimido[1,2-a]benzimidazol-2(10H)-one **13***

$POCl_3$ (0.18 mL, 2.0 mmol) was added to an ice-cooled solution of compound **12** (0.45 g, 1.50 mmol) in 10 mL of 1,2-dichloroethane. The mixture was stirred at room temperature for 45 min, then refluxed for 6 h. The mixture was then treated with an aqueous solution (5 g/20 mL) of $CH_3COONa\cdot 3H_2O$, refluxing for further 45 min. The mixture was then exhaustively extracted with CH_2Cl_2. The combined extracts, dried over Na_2SO_4, afforded an oily residue which was chromatographed on a silica gel column eluting with CH_2Cl_2/EtOAc (1:1) to remove some impurities, then with the mixture acetone–EtOAc–MeOH (9:9:2). The eluate collected, after removal of solvent, gave a thick oil from which, after treatment with a small amount of EtOAc, pure compound **13** separated out as a crystalline solid (0.24 g, 56%); white crystals, m.p. 186–187 °C (EtOAc). IR (KBr, cm^{-1}): 1651 and 1620 s (CO), 1604, 1543. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.16 [t, J = 7 Hz, 6H, N(CH$_2$CH_3)$_2$], 1.44 (t, J = 7 Hz, 3H, NCH$_2$CH_3), 3.18 and 3.25 [2q, J = 7 Hz, 2H + 2H, N(CH_2CH$_3$)$_2$], 4.31 (q, J = 7 Hz, 2H, NCH_2CH$_3$), 5.87 (s, 1H, H-3), 7.19–7.44 (m, 3H, H-7,8,9), 7.92 (m, J_o = 7.8 Hz, 1H, H-6). ^{13}C NMR (75.5 MHz, $CDCl_3$, ppm): δ 10.70, 13.17, 37.03, 44.90, 100.49, 109.13, 114.73, 122.24, 125.12, 125.67, 131.00, 150.28, 152.02, 171.88. MS (ESI) m/z: monoisotopic expected MW = 284.16, experimental full scan m/z [M + H]$^+$ = 285.25. Fragment ions MS^2 [M + H]$^+$ 285.25: m/z 257.17 (84%) ([M + H]$^+$ − CH$_2$=CH$_2$), 241.17 (48%), 162.17 (100%), 124.00 (30%). Anal. $C_{16}H_{20}N_4O$ (C, H, N).

*6.1.7. 10-Ethyl-2-(diethylamino)pyrimido[1,2-a]benzimidazol-4(10H)-one (**6p**)*

A mixture of 2-chloro-10-ethylpyrimido[1,2-*a*]benzimidazol-4(10*H*)-one (**10a**) (0.37 g, 1.5 mmol) and a large excess of diethylamine (5 mL) in ethanol (25 mL) was refluxed for 16 h. The resulting solution was evaporated to dryness in vacuo and the residue was partitioned between CH_2Cl_2 and aq 5% $NaHCO_3$. The combined extracts, dried over Na_2SO_4, afforded an oily residue which was taken up in a little diisopropyl ether to give pure compound **6p** as a crystalline solid (0.38 g, 89%); white crystals, m.p. 152–153 °C (EtOAc/petroleum ether). IR (KBr, cm^{-1}): 1671 s (CO), 1615, 1594, 1538. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): δ 1.22 [t, J = 7 Hz, 6H, N(CH$_2$CH_3)$_2$], 1.42 (t, J = 7 Hz, 3H, NCH$_2$CH_3), 3.52 [q, J = 7 Hz, 4H, N(CH_2CH$_3$)$_2$], 4.20 (q, J = 7 Hz, 2H, NCH_2CH$_3$), 5.19 (s, 1H, H-3), 7.18–7.36 (m, 3H, H-7,8,9), 8.54 (m, J_o = 7.8 Hz, 1H, H-6). ^{13}C NMR (75.5 MHz, $CDCl_3$, ppm): δ 13.06, 13.24, 36.79, 43.05, 77.26, 107.81, 116.08, 121.97, 124.42, 126.34, 130.40, 148.18, 161.18, 161.28. MS (ESI) m/z: monoisotopic expected MW = 284.16, experimental full scan m/z [M + H]$^+$ = 285.25. Fragment ions MS^2 [M + H]$^+$ 285.25: m/z 257.17 (86%) ([M + H]$^+$ − CH$_2$=CH$_2$), 214.08 (18%), 188.08 (100%), 162.08 (56%). Anal. $C_{16}H_{20}N_4O$ (C, H, N).

6.2. Biology

6.2.1. Platelet aggregation

Human blood from healthy volunteers was added to a 130 mM trisodium citrate aqueous solution (volume ratio 9:1), then centrifuged at 100 *g* for 30 min to give platelet-rich plasma (PRP).

PRP (500 μL) was preincubated at 37 °C for 2 min with solvent (dimethylsulfoxide, 5 μL) or drug solution before the addition of the platelet aggregation agent. PRP aggregation was induced by ADP (Sigma) (final concentration 5.0 μM), collagen from bovine tendon (Mascia Brunelli) at the final concentration of 5.0 μg/mL, or A23187 (Sigma) (final concentration 20.0 μM). Before each experiment the stock solutions of ADP, collagen, and A23187 were diluted in saline. Platelet aggregation, performed in an Aggrecoder PA-3210 aggregometer (A. Menarini, Florence, Italy), was measured following the Born's turbidimetric method [33] and quantified by the light transmission reached after 3 min.

6.2.2. Calculation of inhibition

In order to calculate the percentage of inhibition, the extent of aggregation measured in the presence of the compounds tested was always compared with that measured for a control sample containing the solvent, in an experiment carried out under the same conditions. From each series of experiments, in which the inhibitors were tested in at least five concentrations, a percentage inhibition–concentration curve was derived. From this curve the IC_{50} value was calculated as the concentration of inhibitor causing a 50% inhibition of the aggregation. The IC_{50} values reported in Table 1 are averages (±standard deviation) of those obtained from at least five different batches of platelets (usually 5–8 batches).

6.3. Molecular modelling

Compounds **2a**, **2c**, **6i** and **6o** were built, parameterized (Gasteiger–Huckel method) and energy minimized within MOE using MMFF94 forcefield (MOE: Chemical Computing Group Inc. Montreal. H3A 2R7 Canada. http://www.chemcomp.com).

Successively docking studies were performed, using the X-ray data coordinates of the PDE3B isoform (pdb code 1SO2, resolution 2.40 Å) [22]. Each compound was docked into the PDE3 catalytic site, by means of the Surflex docking module implemented in Sybyl-X1.0 (Sybyl-X1.0. Tripos Inc 1699 South Hanley Road. St Louis. Missouri. 63144. USA). Surflex-Dock scores were expressed in −log10(Kd) units to represent binding affinities.

The reliability of the docking protocol was verified by docking simulations of the reference compound, dihydropyridazinone inhibitor Merck 1, which resulted to be in agreement with the experimental X-ray data.

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References

[1] M. Mazzei, A. Balbi, G. Roma, M. Di Braccio, G. Leoncini, E. Buzzi, M. Maresca, Eur. J. Med. Chem. 23 (1988) 237–242.
[2] M. Di Braccio, G. Roma, G. Leoncini, M. Poggi, Il Farmaco 50 (1995) 703–711.
[3] G. Roma, M. Di Braccio, A. Carrieri, G.C. Grossi, G. Leoncini, M.G. Signorello, A. Carotti, Bioorg. Med. Chem. 11 (2003) 123–138.
[4] M. Di Braccio, G.C. Grossi, G. Roma, M.G. Signorello, G. Leoncini, Eur. J. Med. Chem. 39 (2004) 397–409.
[5] G. Roma, M. Di Braccio, G.C. Grossi, D. Piras, G. Leoncini, D. Bruzzese, M.G. Signorello, P. Fossa, L. Mosti, J. Med. Chem. 50 (2007) 2886–2895.
[6] G. Roma, M. Di Braccio, G. Leoncini, B. Aprile, Il Farmaco 48 (1993) 1225–1238.
[7] M. Di Braccio, G. Roma, G. Leoncini, Eur. J. Med. Chem. 30 (1995) 27–38.
[8] G. Roma, N. Cinone, M. Di Braccio, G.C. Grossi, G. Leoncini, M.G. Signorello, A. Carotti, Bioorg. Med. Chem. 8 (2000) 751–768.
[9] K.M. Pritchard, J. Al-Rawi, C. Bradley, Eur. J. Med. Chem. 42 (2007) 1200–1210.
[10] S. Ihmaid, J. Al-Rawi, C. Bradley, Eur. J. Med. Chem. 45 (2010) 4934–4946.
[11] G. Leoncini, M. Maresca, C. Colao, E. Buzzi, M. Mazzei, A. Balbi, Pharmacol. Res. 23 (1991) 139–148.
[12] G. Leoncini, M.G. Signorello, G. Roma, M. Di Braccio, Biochem. Pharmacol. 53 (1997) 1667–1672.
[13] G. Leoncini, M.G. Signorello, D. Bruzzese, M. Di Braccio, G.C. Grossi, G. Roma, Biochem. Pharmacol. 67 (2004) 911–918.
[14] A.R. Katritzky, A.J. Waring, J. Chem. Soc. (1962) 1544–1548.
[15] I. Hermecz, Z. Mészáros, in: A.R. Katritzky (Ed.), Advance in Heterocyclic Chemistry, vol. 33, Academic Press, Inc., New York, NY, 1983, pp. 259–263.
[16] N. Thorup, O. Simonsen, Acta Crystallogr. Sect. C Cryst. Struct. Commun. C41 (1985) 472–474. Chem. Abstr., 102, (1985) 123522d.
[17] I. Hermecz, A. Horváth, L. Vasvári-Debreczy, Z. Mészáros, Synthesis (1984) 152–158.
[18] R.A. Glennon, R.G. Bass, E. Schubert, J. Heterocycl. Chem. 16 (1979) 903–907.
[19] K. Goto, JPN Kokai Tokkyo Koto JP 032215488, A19910920 (20 Sep 1991), Chem. Abstr. 116 (1992) 128962w.
[20] H.R. Snyder, M.M. Robison, J. Am. Chem. Soc. 74 (1952) 4910–4916.
[21] J.J. Wade, R.F. Hegel, C.B. Toso, J. Org. Chem. 44 (1979) 1811–1816.
[22] G. Scapin, S.B. Patel, C. Chung, J.P. Varnerin, S.D. Edmondson, A. Mastracchio, E.R. Parmee, S.B. Singh, J.W. Becker, L.H. Van Der Ploeg, M.R. Tota, Biochemistry 43 (2004) 6091–6100.
[23] X. Guida, H. Jianhua, L. Xiaomin, Eur. J. Med. Chem. 41 (2006) 1080–1083.
[24] A.M. Simonov, A.F. Pozharskii, Zh. Obshch. Khim. 31 (1961) 3970–3974.
[25] A.M. Simonov, A.F. Pozharskii, Zh. Obshch. Khim. 34 (1964) 1572–1574.
[26] H.L. Yale, J.H. Bristol, J. Heterocycl. Chem. 15 (1978) 505–507.
[27] D. Spinks, H.B. Ong, C.P. Mpamhanga, E.J. Shanks, D.A. Robinson, I.T. Collie, K.D. Read, J.A. Frearson, P.G. Wyatt, R. Brenk, A.H. Fairlamb, I.H. Gilbert, ChemMedChem 6 (2011) 302–308.
[28] L. Dalla Via, O. Gia, S. Marciani Magno, A. Da Settimo, A.M. Marini, G. Primofiore, F. Da Settimo, S. Salerno, Il Farmaco 56 (2001) 159–167.
[29] L.N. Divaeva, T.A. Kuzmenko, A.S. Markovnik, V.N. Komissarov, Chem. Heterocycl. Compd. 42 (2006) 463–468.
[30] S.T. Furrer, T.S. McCluskey, PCT Int. Appl. (2009). WO 2009089641 A1 20090723 (23 Jul 2009); US 20100297038 A1 (25 Nov 2010), 1–6.
[31] L.B. Dashkevich, E.S. Korbelainen, Khim. Geterosikl. Soedin. (1966) 602–604.
[32] G.A. Chalyi, V.P. Chernykh, E.V. Koroleva, E.L. Snitkovskij, V.V. Pichugin, V.K. Prusachenko, Russ. RU 2042679, (Cl. C07D487/04) (27 Aug 1995), Chem. Abstr., 124 (1996) 279197s.
[33] G.V.R. Born, Nature (London) 194 (1962) 927–929.