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# Synthesis and molecular modeling of 1*H*-pyrrolopyrimidine-2,4-dione derivatives as ligands for the $\alpha_1$-adrenoceptors

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ABSTRACT

Three different series of 1*H*-pyrrolopyrimidine-2,4-dione derivatives were designed and synthesized as ligands for the $\alpha_1$-adrenergic receptors ($\alpha_1$-ARs). A microwave-assisted protocol was developed in order to improve purity and yields of some final products. The majority of the synthesized compounds, tested in binding assays, displayed $\alpha_1$-AR affinities in the nanomolar range. Highest affinity values were found in derivatives **10b** and **10c** ($K_i$ = 1.4 nM for both) whereas compound **10e** was endowed with the best profile in term of $\alpha_1$-AR affinity ($K_i$ = 2.71 nM) coupled with high selectivity towards 5-$HT_{1A}$ receptors ($K_i$ >10,000). Molecular docking studies were performed on human $\alpha_1$-ARs and human 5-$HT_{1A}$ receptors in order to rationalize the observed experimental affinity and selectivity; these computational studies helped to clarify molecular requirements for the design of high-selective $\alpha_1$-adrenergic ligands.



## 1. Introduction

The adrenergic system is an essential regulator of neuronal, endocrine, cardiovascular, and metabolic functions. Adrenergic receptors (ARs) are endogenously activated by adrenaline and noradrenaline and are members of the G-protein coupled receptors (GPCRs) super-family that transmit their signal across the plasma membrane. All ARs are characterised by a conserved intra-membrane framework formed by seven membrane-spanning α-helices (TM1-7).[1,2] The ARs are divided into three main classes ($\alpha_1$, $\alpha_2$, β) and, within each family, they are further divided into three subtypes: $\alpha_1$ ($\alpha_{1A}$, $\alpha_{1B}$, $\alpha_{1D}$) having high affinity for prazosin, $\alpha_2$ ($\alpha_{2A}$, $\alpha_{2B}$, $\alpha_{2C}$), and β ($\beta_1$, $\beta_2$, $\beta_3$).[1,2]

In the past few years, the efforts to design ligands selective for each $\alpha_1$-AR subtypes and to discover selective $\alpha_1$-AR ligands with respect to other related GPCRs proceeded at the same rate and continue to represent an area of active research.[3–5] The main reason for such interest is the wide distribution of $\alpha_1$-ARs in peripheral tissues and in CNS and their involvement in a number of diseases such as hypertension, myocardial hypertrophy, benign prostatic hyperplasia, and lower urinary tract symptoms (LUTS).[3–5] Besides these diseases, $\alpha_1$-ARs seem to be involved also in modulation of stress-induced anxiety-like behavioral responses, in control of motor activity (e.g., a recent report demonstrated that, in rat, a CNS-penetrating $\alpha_1$-AR antagonist can reduce L-DOPA-induced dyskinesia), in regulation of neurogenesis and gliogenesis, in growth of small mouse cholangiocytes;[6–8] $\alpha_1$-ARs of the locus coeruleus (LC) seem to be implicated in behavioral activation in novel surroundings.[9,10] Another interesting finding is that $\alpha_{1A}$-ARs stimulation is capable of influencing the balance of human ether-a-go-go-related gene (HERG) potassium channel synthesis and degradation via multiple signaling pathways, a process that may have relevance in cardiac diseases and treatment.[11] Recently, it was reported that stimulation of noradrenergic $\alpha_1$-ARs is involved in nicotine self-administration and relapse, possibly via facilitation of nicotine-induced activation of the mesolimbic dopaminergic system; the findings point to $\alpha_1$-ARs blockade as a potential new approach to the treatment of tobacco dependence in humans.[12] Moreover $\alpha_1$- along with $\alpha_2$-adrenergic receptors seem to play an important role in scopolamine-induced amnesia and scopolamine state-dependent memory and exert opposing effects on excitability of main olfactory bulb granule cells.[13,14] All these recent findings

*Abbreviations:* 5-$HT_{1A}$, 5-serotonin$_{1A}$; ARs, adrenergic receptors; $\alpha_1$-ARs, $\alpha_1$-adrenergic receptors; GPCRs, G-protein coupled receptors; HBA, hydrogen bond acceptor; $MLP_{InS}$, molecular lipophilicity potential interaction score; RN5, 3-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-1*H*-pyrimido[5,4-*b*]indole-2,4(3*H*,5*H*)-dione; TEA, triethylamine; TM, transmembrane domain; VIF, variance inflaction factor.

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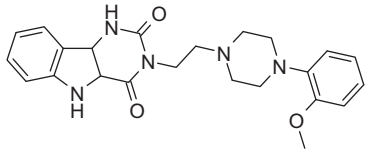
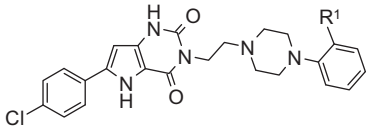
still makes $\alpha_1$-ARs a fascinating target for the development of novel selective ligands, agonist, antagonists, pharmacological tools, and future therapeutic agents.

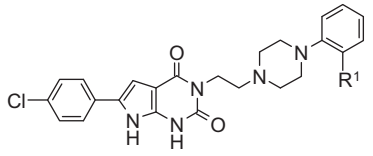
For many years, our research group has focused on a project aimed at investigating new adrenergic ligands based on an arylpiperazinyl moiety, a well known pharmacophore present in several class of $\alpha_1$-AR ligands. We described both $\alpha_1$-AR ligands selective over other related receptors such as serotoninergic 5-$HT_{1A}$ and ligands selective among different $\alpha_1$-AR subtypes.[15–22] RN5 (Fig. 1) being highly selective for $\alpha_1$-ARs with respect to other related GPCRs, is one of the most interesting derivatives identified, to date, by our research group.[15] RN5 served as lead compound for several structural modifications performed on different portions of the molecule: the arylpiperazinyl moiety, the tricyclic system, and the connecting alkyl chain. In particular, we have previously demonstrated that, by fixing a 2-chloro or 2-methoxyphenylpiperazinyl residue, structural modifications on the tricyclic system afforded highly selective $\alpha_1$-AR ligands over the related GPCRs. Such as an example the replacement of the pyrrole nucleus in the tricyclic ring with a furane or thiophene residue slightly impaired affinity and/or selectivity, whereas, when the indole nucleus of RN5 was substituted by an arylpyrrolo moiety, noteworthy results were obtained.[16–19] In particular, two series of isomeric compounds were described and representative compounds **1a–b** and **2a–b** are depicted in Figure 1.[18,19] The data collected from structure–affinity relationship (SAR) studies on these two series suggested that the presence of an arylpyrrolo moiety coupled with a 2-methoxyphenylpiperazinyl moiety (e.g., **1a** and **2a**), afforded $\alpha_1$-AR ligands with high-affinity, albeit selectivity data over dopaminergic $D_1$ and $D_2$ receptors were not always satisfactory. Conversely, as general trend, derivatives in which $R^1$ is a chlorine atom (e.g., **1b** and **2b**), showed high $\alpha_1$-AR affinity and excellent selectivity with respect to 5-$HT_{1A}$ and dopaminergic $D_1$ and $D_2$ receptors (Fig. 1).



**RN5** $\alpha_1$ $K_i$ = 0.21 nM, 5-$HT_{1A}$ $K_i$ = 50 nM



**1a** $R^1$ = $CH_3O$; $\alpha_1$ $K_i$ = 7.68 nM, 5-$HT_{1A}$ $K_i$ = > 10000 nM
**1b** $R^1$ = Cl; $\alpha_1$ $K_i$ = 11.70 nM, 5-$HT_{1A}$ $K_i$ = > 10000 nM



**2a** $R^1$ = $CH_3O$; $\alpha_1$ $K_i$ = 4.08 nM, 5-$HT_{1A}$ $K_i$ = 401.3 nM
**2b** $R^1$ = Cl; $\alpha_1$ $K_i$ = 2.64 nM, 5-$HT_{1A}$ $K_i$ = 1513 nM

**Figure 1.** Structures of known $\alpha_1$-AR ligands **RN5**, **1a–b**, **2a–b**.

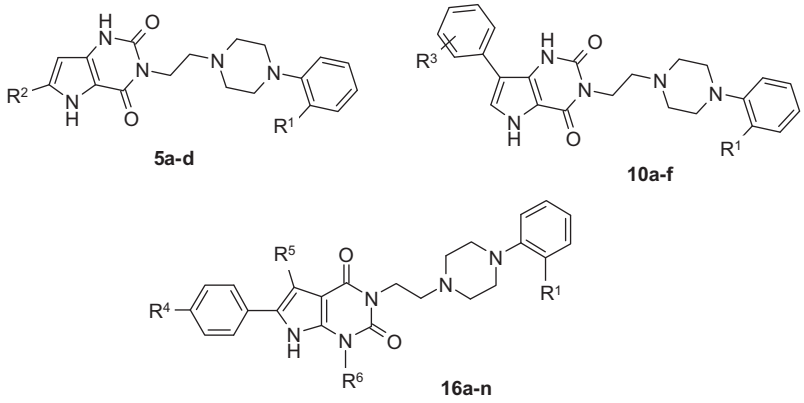
On the basis of our previous work and taking into account the excellent binding profile of both classes of derivatives, in this paper we report on further structural modifications on the two previously synthesized series (Fig. 1), with the aim of getting a new insight into the structural requirements that direct selectivity toward $\alpha_1$-ARs. Three series of compounds (Fig. 2) were designed in which the role of the aryl residue linked to the two isomeric pyrrolopyrimidinedione scaffolds was mainly investigated. In the first series, the phenyl ring at the 6-position of 1*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione nucleus was replaced with a 2-furanyl or 1,1-dimethylethyl residues (**5a–d**). In the second series, the phenyl ring was moved from the 6-position to the 7-position of the 1*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione nucleus (**10a–f**). In the third series of derivatives (**16a–n**), it was investigated how a phenyl ring, linked at different positions of the pyrrolopyrimidinedione scaffold, influenced the binding profile.

All the novel synthesized compounds were tested in binding assays and $K_i$ values towards $\alpha_1$-ARs and 5-$HT_{1A}$ receptors are reported. Moreover, molecular docking studies were performed on human $\alpha_1$-ARs and human 5-$HT_{1A}$ receptors in order to rationalize the observed experimental affinity and selectivity.

## 2. Chemistry

Novel derivatives were synthesized as reported in Schemes 1–3 and the synthetic routes follow a similar, but slightly modified, strategy with respect to the one used for the preparation of compounds **1** and **2**.[18,19]

Briefly, the appropriate aminosubstitutedpyrrolecarboxylic acid ethyl esters (**3a–b**), prepared according to literature,[23] were reacted with 1-chloro-2-isocyanatoethane in toluene (Scheme 1).



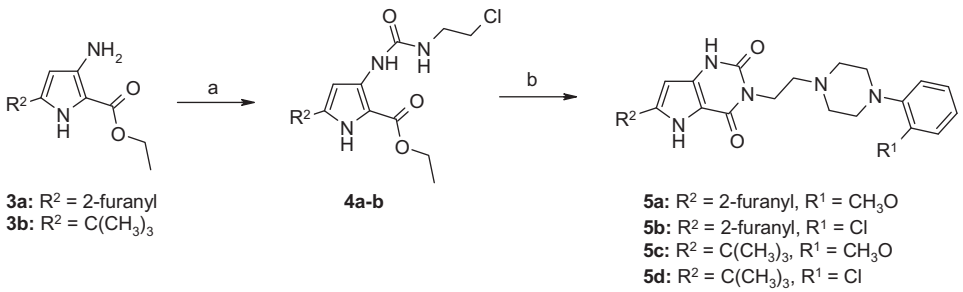
**5a-d** **10a-f** **16a-n**

**Figure 2.** Structure of novel 1*H*-pyrrolopyrimidine-2,4-dione derivatives **5a–d**, **10a–f**, and **16a–n**.

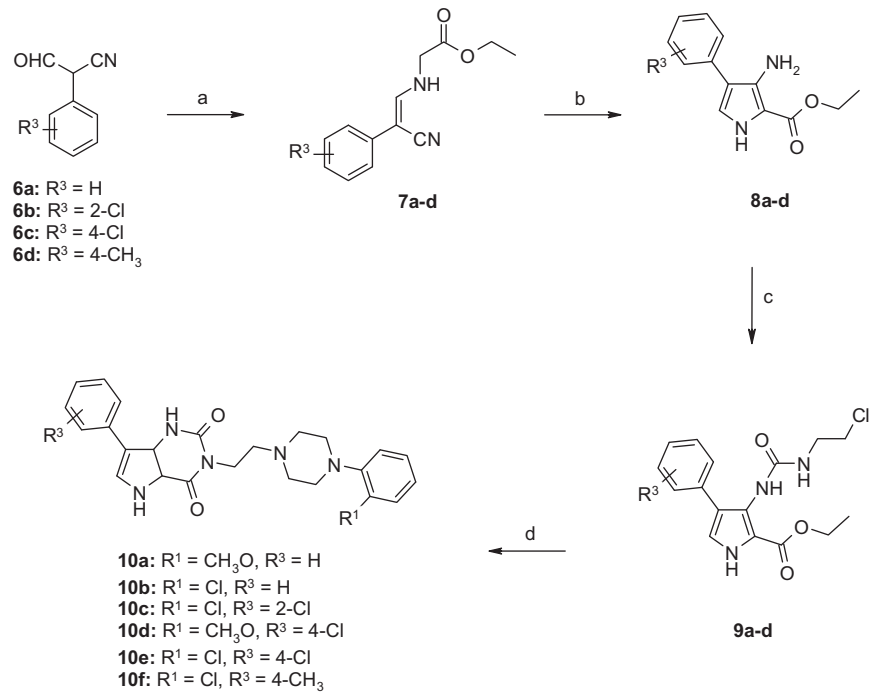
Obtained ureas (**4a**–**b**) were allowed to react with the appropriate 1-(2-substitutedphenyl)piperazine in 2-propanol in the presence of NaI and $NaHCO_3$ at reflux; intermediates were converted, without isolation, into final products (**5a**–**d**) in refluxing methanolic potassium hydroxide (Scheme 1).

For the synthesis of aminoesters **8a**–**d** (Scheme 2) the starting α-formylsubstitutedbenzeneacetonitriles (**6a**–**d**) were reacted with glycine ethyl ester.[24] NMR spectra of the obtained *N*-[2-cyano-2-(substitutedphenyl)ethenyl]glycine ethyl esters (**7a**–**d**) showed no signals which might have been attributed to the alternate iminic structure, as before demonstrated by Lim et al.[25] Compounds **7a**–**d** were cyclized to give the desired aminoesters **8a**–**d** in the presence of sodium in ethanol.[26,27] These latter compounds were allowed to react with 1-chloro-2-isocyanatoethane in refluxing toluene to give intermediate ureas **9a**–**d**, which were converted in one pot into the desired final compounds **10a**–**f** by using the same procedure reported for **5a**–**d** (Scheme 2). However, it was not possible to purify final compounds by washings with water and EtOH and subsequent recrystallization as for compounds **5a**–**d**, so purification by column chromatography was required.

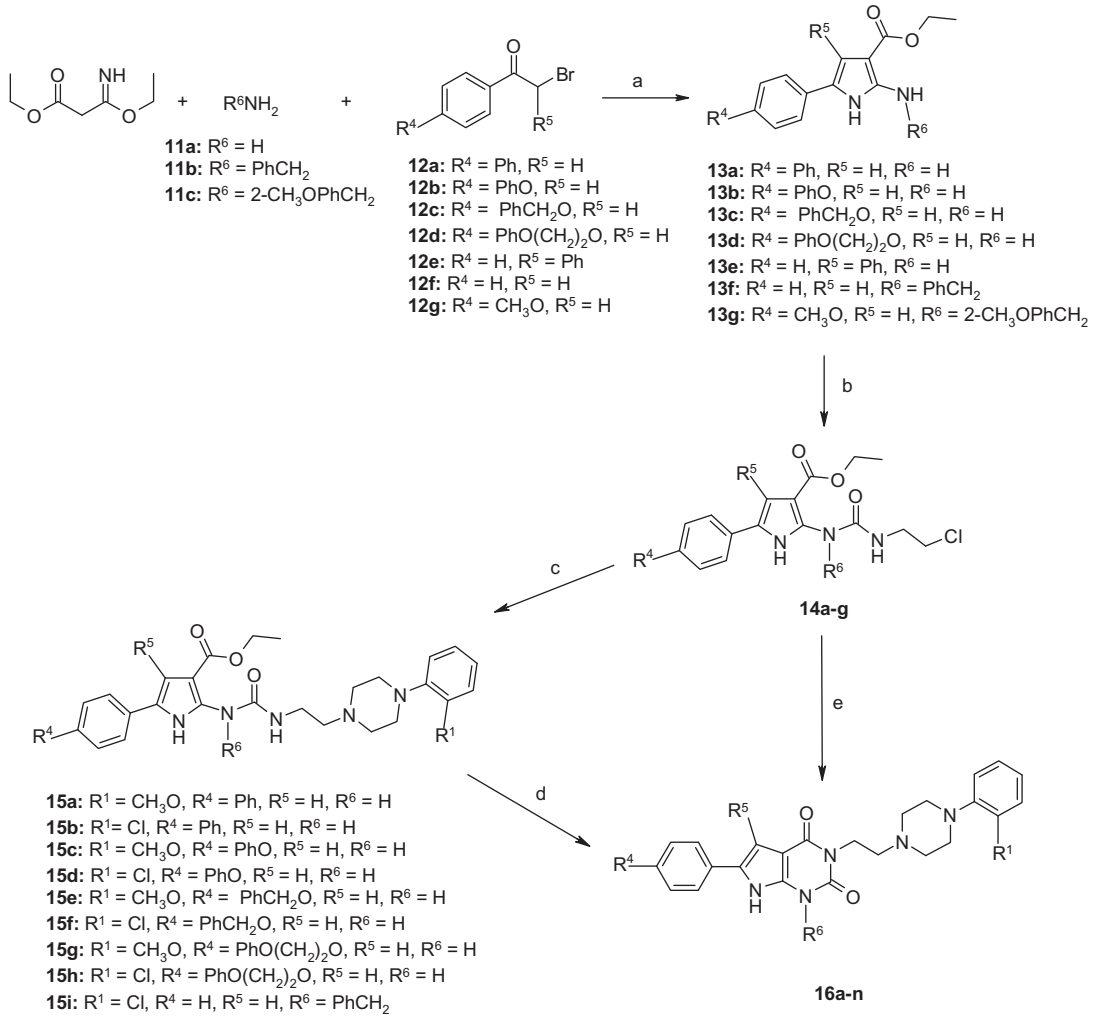
For the synthesis of aminoesters **13a**–**g**,[28–30] the amines **11a–c** were allowed to react with ethyl 3-ethoxy-3-iminopropanoate hydrochloride in the presence of $K_2CO_3$ and ethanol for 24 h at 22 °C and then with the appropriate 2-bromo-1-(4-substitutedphenyl)ethanone, **12a**–**g**,[31,32] at reflux for 2 h (Scheme 3). With respect to preparation of compound **12d,** unknown in the literature, 1-[4-(2-phenoxyethoxy)phenyl]ethanone was brominated in glacial acetic acid with $Br_2$ to give the desired compound which, without isolation, was condensed with 3-amino-3-imino-2-propanoic acid ethyl ester, affording compound **13d**. Aminoesters **13a**–**g** were reacted with 1-chloro-2-isocyanatoethane in refluxing toluene to obtain ureas **14a**–**g** which were allowed to react with the appropriate 1-(2-substitutedphenyl)piperazine in THF in the presence of NaI and $NaHCO_3$ (Scheme 3). Unlike compounds **5a**–**d** and **10a**–**f** and in order to facilitate purification of the final compounds, intermediates **15a**–**i** were isolated and purified by column chromatography (Scheme 3). Alternatively, some intermediates obtained at this stage were dissolved in EtOAc, filtered through a pad of silica gel, concentrated, and used into the final step without any further purification. The ring closure into final products **16a**–**n** was




**Scheme 1.** Synthesis of 3-[2-[4-(2-substitutedphenyl)piperazin-1-yl]ethyl]-6-(2-substituted)-1,5-dihydropyrrolo[3,2-*d*]pyrimidine-2,4-dione **5a**–**d**. Reagents and conditions: (a) $ClCH_2CH_2NCO$, toluene, 22 °C, 16 h; (b) (i) 1-(2-substitutedphenyl)piperazine, $NaHCO_3$, NaI, 2-propanol, reflux, 4 days; (ii) KOH/MeOH, reflux, 2 h.




**Scheme 2.** Synthesis of 3-[2-[4-(2-substitutedphenyl)piperazin-1-yl]ethyl]-7-(substitutedphenyl)-1,5-dihydropyrrolo[3,2-*d*]pyrimidine-2,4-dione **10a**–**f**. Reagents and conditions: (a) $CH_3CH_2OOCCH_2NH_2$, TEA, 4-methylbenzenesulfonic acid, toluene, reflux, 1 h; (b) EtONa, ethanol, reflux, 48 h; (c) $ClCH_2CH_2NCO$, toluene, reflux, 6 h; (d) (i) 1-(2-substitutedphenyl)piperazine, $NaHCO_3$, NaI, THF, reflux, 4 days; (ii) KOH/MeOH, reflux, 2 h.




**Scheme 3.** Synthesis of 3-[2-[4-(2-substitutedphenyl)-1-piperazinyl]ethyl]-1,5,6-substituted-1*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione **16a–n**. Reagents and conditions: (a) (i) $K_2CO_3$, ethanol, 22 °C, 24 h; (ii) **12a–g**, $K_2CO_3$, reflux, 2 h; (b) $ClCH_2CH_2NCO$, toluene, reflux, 6 h; (c) 1-(2-substitutedphenyl)piperazine, $NaHCO_3$, NaI, THF, reflux, 24 h; (d) KOH/MeOH, microwave irradiation, 120 °C, 15 min, 200 W, 150 psi, sealed tube; (e) (i) 1-(2-substitutedphenyl)piperazine, $NaHCO_3$, NaI, THF, reflux, 24 h; (ii) KOH/MeOH, microwave irradiation, 120 °C, 15 min, 200 W, 150 psi, sealed tube.

achieved in methanolic potassium hydroxide. A microwave-assisted protocol for the final ring closure was developed to improve purity and yields of the final products. Under microwave irradiation (120 °C, 15 min, 200 W, 150 psi, sealed tube), it was possible to obtain the desired final compounds **16a–n** in an average yield of 69%.

## 3. Pharmacology

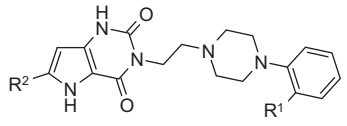
New 1*H*-pyrrolopyrimidine-2,4-dione derivatives **5a–d**, **10a–f** and **16a–n** were evaluated in binding assays on rat cortex for $\alpha_1$-ARs using [$^3$H]prazosin as radioligand and rat hippocampus for 5-$HT_{1A}$ using [$^3$H]-8-OH-DPAT. Their affinity values, expressed as $K_i$, are summarized in Tables 1–3.

## 4. Results and discussions

### 4.1. Structure–activity relationship studies

New synthesized ligands **5a–d**, **10a–f**, and **16a–n** can be regarded as constituted by three different regions, each one of them systematically modified: (i) a 'central core' represented by the two isomeric pyrrolopyrimidinedione nuclei identified in our previous works as excellent scaffolds for molecules with high affinity and/or selectivity towards $\alpha_1$-ARs; (ii) a 'side pharmacophoric portion'

**Table 1**
$\alpha_1$-ARs and 5-$HT_{1A}$ receptors binding data for compounds **5a–d**



| Compd | $R^2$ | $R^1$ | $K_i$[a] (nM) | | 5-$HT_{1A}$/$\alpha_1$[b] |
|---|---|---|---|---|---|
| | | | $\alpha_1$ | 5-$HT_{1A}$ | |
| **5a** | 2-Furanyl | $OCH_3$ | 3.38 ± 0.33 | 28.85 ± 1.86 | 8.54 |
| **5b** | 2-Furanyl | Cl | 14.17 ± 2.01 | 219.54 ± 24.43 | 15.50 |
| **5c** | $C(CH_3)_3$ | $OCH_3$ | 1.69 ± 0.23 | 46.73 ± 7.14 | 27.7 |
| **5d** | $C(CH_3)_3$ | Cl | 1.39 ± 0.005 | 65.15 ± 12.99 | 46.9 |
| **1a**[c] | | | 7.68 ± 1.63 | >10,000 | >1000 |
| **1b**[c] | | | 11.70 ± 2.17 | >10,000 | >800 |

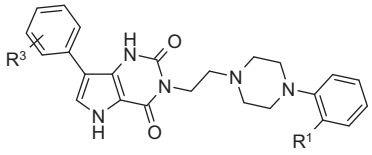
[a] $K_i$ values were calculated as described in the Section 6 and are the mean ± SD of three separate experiments.
[b] Ratio $K_i$ 5-$HT_{1A}$/$K_i$ $\alpha_1$.
[c] Data taken from Ref. 18.

represented by the well known 2-chloro or 2-methoxyphenylpiperazine moieties; (iii) a 'second side region', usually represented by one or more phenyl/aryl rings linked at different positions of the central pyrrolopyrimidinedione core. Noteworthy, binding results indicated that the majority of the compounds described herein showed preferential affinity for the $\alpha_1$-ARs ($K_i$ values in the nanomolar range) with respect to the 5-$HT_{1A}$ receptors.

The first series of derivatives **5a–d** (Table 1) is strightly related to **1a–b**. In these compounds, the 1,5-dihydropyrrolo[3,2-*d*]-pyrimidine-2,4-dione nucleus was fixed as 'central core' and the 2-chloro or 2-methoxyphenylpiperazine moieties were maintained as the 'side pharmacophoric portion'. The 'second side region' was modified with respect to the parent compounds **1a–b** by introducing 2-furanyl or 1,1-dimethylethyl residues at the 6-position of the pyrrolopyrimidinedione nucleus, in order to further investigate the role of the 'second side region' on affinity and selectivity. With reference to binding data, compounds **5a–d** showed nanomolar affinity for $\alpha_1$-ARs but, unfortunately, their selectivity over 5-$HT_{1A}$ receptors was lower when compared to the parent compounds **1a–b**.

The second series **10a–f** (Table 2) was also designed and synthesized with the aim to better understand the contribution of the 'second side region' and specifically the role of the aryl ring; in fact in this series the phenyl ring was moved from the 6-position (**1a–b**) to the 7-position of the 1,5-dihydropyrrolo[3,2-*d*]pyrimidine-2,4-dione scaffold again fixed as 'central core'. As a general trend, **10a–f** showed the highest affinity values at $\alpha_1$-ARs coupled with good selectivity towards 5-$HT_{1A}$ receptors in the whole set of synthesized compounds. The influence of the substitutions on the phenyl ring (second side region) linked to the heterobicyclic system (central core) on affinity and/or selectivity was investigated by introducing a methyl or a chlorine residue at the 2- or the 4-position, according to previous work. This structural modification while maintaining affinity at $\alpha_1$-ARs in the low nanomolar range, allowed a modulation of the selectivity with respect to 5-$HT_{1A}$ receptors. Analyzing binding data reported in Table 2, it can be observed that the unsubstituted phenyl derivatives **10a–b** gained affinity towards $\alpha_1$-ARs when compared to the parent compounds **1a–b** albeit a loss of selectivity over the 5-$HT_{1A}$ receptors was observed. However, binding data for **10a–b** were encouraging enough for further exploration. The introduction of a chlorine at the 2-position of phenyl ring afforded compound **10c** which maintained $\alpha_1$-AR affinity and gained two-fold selectivity with respect to the parent unsubstituted **10b**. In compounds **10d–e** the chlorine was

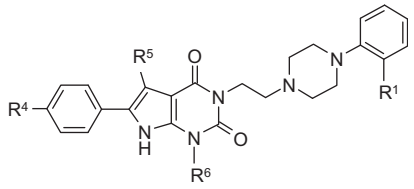
**Table 2**
$\alpha_1$-ARs and 5-$HT_{1A}$ receptors binding data for compounds **10a–f**



| Compd | $R^3$ | $R^1$ | $K_i$[a] (nM) | | 5-$HT_{1A}/\alpha_1$[b] |
|---|---|---|---|---|---|
| | | | $\alpha_1$ | 5-$HT_{1A}$ | |
| **10a** | H | $OCH_3$ | 2.08 ± 0.42 | 13.40 ± 1.28 | 6.44 |
| **10b** | H | Cl | 1.14 ± 0.11 | 43.10 ± 3.04 | 37.8 |
| **10c** | 2-Cl | Cl | 1.14 ± 0.27 | 81.51 ± 12.22 | 71.5 |
| **10d** | 4-Cl | $OCH_3$ | 3.36 ± 0.16 | 32.61 ± 8.19 | 9.70 |
| **10e** | 4-Cl | Cl | 2.71 ± 0.41 | >10,000 | >3500 |
| **10f** | 4-$CH_3$ | Cl | 4.96 ± 0.02 | >10,000 | >2000 |
| **1a**[c] | | | 7.68 ± 1.63 | >10,000 | >1000 |
| **1b**[c] | | | 11.70 ± 2.17 | >10,000 | >800 |

[a] $K_i$ values were calculated as described in the Section 6 and are the mean ± SD of three separate experiments.
[b] Ratio $K_i$ 5-$HT_{1A}/K_i$. $\alpha_1$.
[c] Data taken from Ref. 18.

**Table 3**
$\alpha_1$-ARs and 5-$HT_{1A}$ receptors binding data for compounds **16a–n**



| Compd | $R^4$ | $R^5$ | $R^6$ | $R^1$ | $K_i$[a] (nM) | | 5-$HT_{1A}/\alpha_1$[b] |
|---|---|---|---|---|---|---|---|
| | | | | | $\alpha_1$ | 5-$HT_{1A}$ | |
| **16a** | Ph | H | H | $OCH_3$ | 54.30 ± 8.54 | 3680 ± 869 | 67.8 |
| **16b** | Ph | H | H | Cl | 183 ± 47 | >10,000 | 54.6 |
| **16c** | PhO | H | H | $OCH_3$ | 43.71 ± 10.4 | 17.10 ± 0.69 | 0.39 |
| **16d** | PhO | H | H | Cl | 125 ± 17 | 4720 ± 387 | 37.8 |
| **16e** | $PhCH_2O$ | H | H | $OCH_3$ | 10.31 ± 1.53 | 784 ± 207 | 76.0 |
| **16f** | $PhCH_2O$ | H | H | Cl | 70.91 ± 1.37 | >10,000 | 141 |
| **16g** | $PhO(CH_2)_2O$ | H | H | $OCH_3$ | 53.63 ± 3.27 | >10,000 | 186 |
| **16h** | $PhO(CH_2)_2O$ | H | H | Cl | 126 ± 16 | >10,000 | 79 |
| **16i** | H | Ph | H | $OCH_3$ | 4.82 ± 0.80 | 466 ± 65 | 96.7 |
| **16k** | H | Ph | H | Cl | 11.90 ± 3.01 | 1540 ± 389 | 129 |
| **16j** | H | H | $PhCH_2$ | $OCH_3$ | 24.50 ± 1.52 | 198 ± 24 | 8.08 |
| **16l** | H | H | $PhCH_2$ | Cl | 35.41 ± 1.66 | 1020 ± 25 | 28.8 |
| **16m** | $CH_3O$ | H | 2-$CH_3OPhCH_2$ | $OCH_3$ | 15.86 ± 1.03 | 184 ± 47 | 11.6 |
| **16n** | $CH_3O$ | H | 2-$CH_3OPhCH_2$ | Cl | 89.20 ± 13.0 | 4670 ± 1333 | 52.3 |
| **2a** | | | | | 4.08 ± 0.31 | 401.3 ± 26.3 | 98.4 |
| **2b** | | | | | 2.64 ± 0.25 | 1513 ± 172.8 | >500 |

[a] $K_i$ values were calculated as described in the Section 6 and are the mean ± SD of three separate experiments.
[b] Ratio $K_i$ 5-$HT_{1A}/K_i$. $\alpha_1$.
[c] Data taken from Ref. 19.

shifted from the 2- to the 4-position of the phenyl ring fixed as 'second side region'. In particular, compound **10d** maintained affinity values for $\alpha_1$-ARs comparable or slightly improved respect to the parents **1a** and **10a**, whereas a loss of selectivity was observed (5-$HT_{1A}/\alpha_1$ **1a** >1000; **10a** = 6.44; **10d** = 9.70). Compound **10e**, (Table 2, $R^1$ = Cl), exhibited 4.3-fold higher affinity ($K_i$ = 2.71 nM) than parent **1b** towards $\alpha_1$-ARs coupled with high selectivity with respect to 5-$HT_{1A}$ receptors (5-$HT_{1A}/\alpha_1$ **1b** >800; **10d** = 9.70; **10e** >3500). On the basis of this data, we decided to synthesize **10f**, in which a 4-methylphenyl residue was chosen as 'second side region' and was coupled with a 2-chlorophenylpiperazine moiety as 'side pharmacophoric portion'. This compound, which exhibited a good binding profile ($\alpha_1$-AR $K_i$ = 4.96 nM, 5-$HT_{1A}/\alpha_1$ >2000), along with **10e** represent the most interesting derivatives reported herein. These binding results underline that the presence of a 4-substituted phenyl ring as 'second side region' and a 2-chloro substitution on the phenyl ring of the 'side pharmacophoric portion' are crucial to address high affinity and selectivity to $\alpha_1$-ARs, confirming previously observed SARs.[18,19]

On the basis of the good binding profile observed for the previously synthesized **2a–b**, a third series of derivatives was designed by using the isomeric 1*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione nucleus as 'central core' and the 2-chloro or 2-methoxyphenylpiperazine moieties as 'side pharmacophoric portion' (Table 3, compounds **16a–n**). With respect to the 'second side region', a first aryl ring was maintained fixed at the 6-position of the 'central core' and a second one was introduced at different positions ($R^4$, $R^5$, or $R^6$). As a general trend, a slight reduction in affinity and/or selectivity over 5-$HT_{1A}$ was observed as compared to compounds **2a–b**, **5a–d**, and **10a–f**. Nevertheless, some SAR trends could be observed. Generally, with a few exceptions, selectivity over 5-$HT_{1A}$ receptors decreased with respect to **2a–b** and, in the case of **16c**, a binding affinity inversion was observed (5-$HT_{1A}$, $K_i$ = 17.10 nM; $\alpha_1$-AR, $K_i$ = 43.71 nM, 5-$HT_{1A}/\alpha_1$ = 0.39). With regard to the 'side

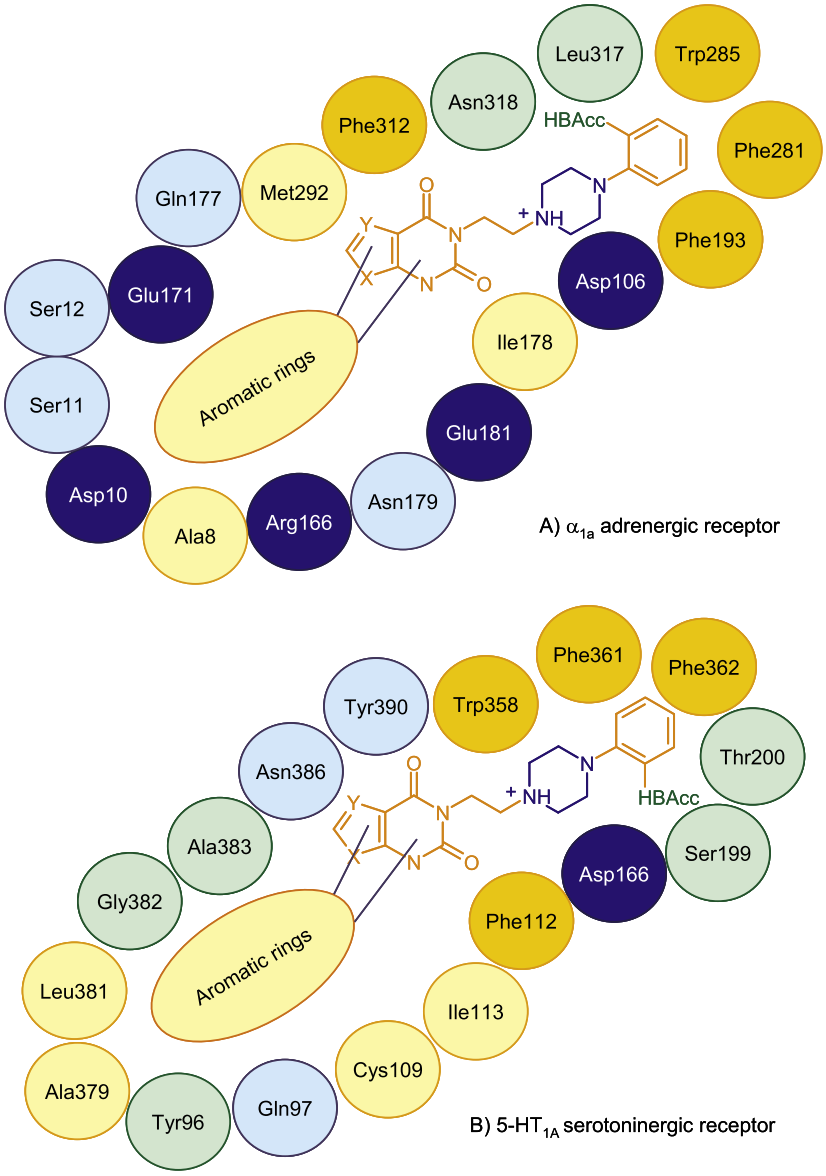



**Figure 3.** 2D scheme showing the main residues of (A) $\alpha_{1a}$-AR and (B) 5-$HT_{1A}$ serotonergic receptors involved in the interaction with the considered compounds. Residues and ligand groups are coloured considering the interaction complementarity. In particular, orange for aromatic π–π stacking, yellow for hydrophobic interaction, green for H-bond acceptors/donors, blue for ionic interactions, and light blue for polar interactions. Leu317, Asn318 of the $\alpha_{1a}$-AR and Gly382, Ala383 of the 5-$HT_{1A}$ receptors are coloured in green because their backbone hydrogens stabilize H-bonds to the ligand.

pharmacophoric portion', when a 2-methoxyphenylpiperazine was used, higher affinities than the 2-chlorosubstituted analogs were observed. With respect to the 'second side region', the introduction of a second aryl or arylmethyl ring linked at 1- or 5-position ($R^6$ and $R^5$, respectively) of the 'central core' afforded compounds **16i–n**. The presence of an arylmethyl residue ($R^6$) at the 1-position of the pyrrolopyrimidinedione nucleus (**16j–n**) was detrimental for adrenergic affinity, whereas the introduction of a phenyl ring ($R^5$) linked at the 5-position of the 'central core' gave compounds **16i–k** endowed with reasonable $\alpha_1$-AR affinity. These last derivatives, **16i–k**, are the most interesting compounds of this series achieving $K_i$ values towards $\alpha_1$-ARs in the low nanomolar range (**16i** $K_i$ = 4.82 nM; **16k** $K_i$ = 11.90 nM) coupled with a 100-fold selectivity with respect to 5-$HT_{1A}$ receptors.

### 4.2. Computational results

All designed compounds (**5a–d**, **10a–f**, and **16a–n**) were docked into $\alpha_{1a}$-AR and 5-$HT_{1A}$ receptor models[33,34] to better understand their binding modes. Among different $\alpha_1$AR subtypes, molecular docking studies were focused on the human $\alpha_{1a}$-AR, mainly for the availability of a well validated model and the very high homology among $\alpha_1$-AR subtypes (always greater than 73% as computed by ClustalX and represented by Fig. S1, Supplementary data). Moreover, docking studies on the human 5-$HT_{1A}$ serotoninergic receptors were carried out to rationalize the observed 5-$HT_{1A}/\alpha_{1a}$ selectivity and to clarify molecular requirements for the design of high-selective $\alpha_1$-AR ligands.

Considering the $\alpha_1$-ARs, a global analysis of the generated complexes for the 24 analyzed ligands (**5a–d**, **10a–f**, and **16a–n** as compiled in Tables 1–3) reveals a significant homogeneity among them, presumably because the ligands share a common structure and all complexes are tethered by a pivotal ion-pair between the piperazine protonated nitrogen of the ligand and the carboxylate group of Asp106.[35] The average distance between the hydrogen bonded to nitrogen and the oxygens of the carboxylate group is about 1.8 Å. This interaction, which is shared also by endogenous ligands, plays a key role for the affinity and is clearly conserved in all $\alpha_1$-AR subtypes. The 2-substituted phenyl bonded at the 4-position of the piperazine ring is inserted in a subcavity lined by aromatic residues (Phe193, Phe281, Trp285), with which it can generate $\pi$–$\pi$ stacking. Noticeably, such aromatic residues are highly conserved in all $\alpha_1$-AR subtypes (as seen in Fig. S1) thus suggesting that the $\pi$–$\pi$ interactions stabilize the complexes in all $\alpha_1$-AR subtypes. Moreover, the 2-substituent (chloro or methoxy) on the 'side pharmacophoric portion' contacts the backbone of Leu317 and Asn318 with which it elicits two H-bonds. In Figure 3A, the main residues of the $\alpha_{1a}$-AR involved in the interaction with compounds **5a–d**, **10a–f**, and **16a–n** are schematized; from Figure 3A, it can be clearly seen that both the pyrrolopyrimidine-2,4-dione systems cannot realize specific polar contacts, but generate apolar interactions with Ile178, Met292 and Phe312. Moreover, Figure 3A clearly suggests that the subcavity which harbours the aromatic residues bound to the pyrrolopyrimidine-2,4-dione systems is flanked by a rich set of polar residues which cannot stabilize significant interactions with such hydrophobic ligand moieties, although the binding site is large enough to include all considered ligands. Calculations of the interaction surface by the use of Molecular Lipophilicity Potential Interaction Score ($MLP_{InS}$)[36] are reported for compounds **16a** and **10e** in Figure 4. These calculations confirm that aromatic rings bound to the pyrrolopyrimidine-2,4-dione systems, particularly for compound **16a**, do not participate to the complex stabilization since they do not match the physicochemical properties of the sub-pocket in which they are accommodated. However, a smaller aromatic portion (compare **10e**, Fig. 4B vs **16a**, Fig. 4A) is better tolerated and induces a clear enhancement of the measured affinity ($\alpha_1$-AR $K_i$ = 2.71 and 54.30 nM, respectively). This result can be explained by considering that unfavourable interactions are probably decreased for compound **10e**. Conversely, extensive modifications of the biphenyl moieties (e.g., compounds **16a–h**) do not induce remarkable variations in the binding affinity.

As for the $\alpha_1$-AR, a similar docking analysis was carried out for the 5-$HT_{1A}$ complexes. For the compounds with a significant affinity towards this receptor (e.g., **5a**, **10a**, and **16c**), the binding mode is very similar as that was found in previous studies[18,34] as

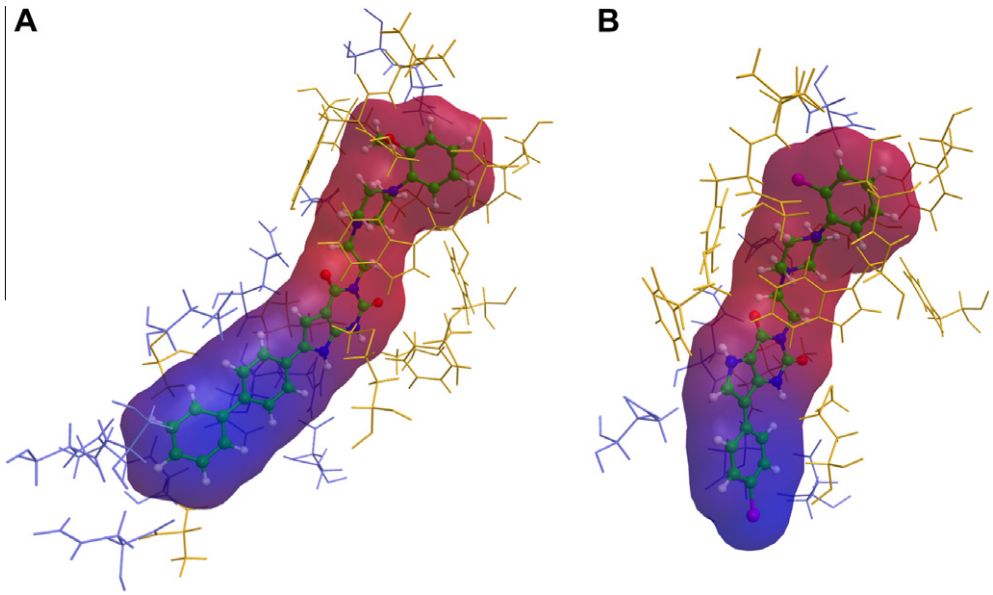



**Figure 4.** $MLP_{InS}$ interaction surface for compound **16a** (A) and **10e** (B). The red area shows ligand groups able to generate favourable interactions with the pocket residues, whereas blue areas show regions with low complementarity with the receptor. The hydrophilic and hydrophobic aminoacids are coloured in azure and orange, respectively. In both cases, although there is a good sterical complementarity between ligand and receptor, the aromatic systems bound to the 1*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione and 1,5-dihydropyrrolo[3,2-*d*]pyrimidine-2,4-dione groups are unable to realize beneficial interactions, because they are surrounded by polar aminoacids. Compound **10e** elicits less repulsive interactions (smaller blue area) than **16a** (larger blue area) thus explaining its better affinity ($pK_i$ = 8.57 vs 7.27).

schematized by Figure 3B. More in detail, the main interaction between the charged nitrogen of the piperazine moiety and Asp166 was observed with an average distance of 1.8 Å. In order to achieve a significant 5-$HT_{1A}$ affinity, a ligand should also possess a suitably arranged HBA function (as in the case of the methoxyl group at the 2-position of the phenyl bound to the piperazine) to contact Ser199; this was confirmed by site-directed mutagenesis studies.[37] Hence, ligands bearing a chlorine instead of the methoxyl group on the 'side pharmacophoric portion' have a modest affinity for the 5-$HT_{1A}$ receptor, preserving a good affinity for the $\alpha_1$-ARs (e.g., compare **10e** vs **10d**). However, the presence of additional interactions with Tyr390 and Asn386 as observed for compounds **10b** and **10c** can partially counterbalance the incapacity to contact Ser199. A second feature, which can influence the observed selectivity, concerns the pocket which harbours the pyrrolo[2,3-*d*]pyrimidine-2,4-dione systems. This pocket appears clearly smaller and less flexible in 5-$HT_{1A}$ receptor when compared to $\alpha_1$AR thus ligands with multiple rings at the 6-position of the 1*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione scaffold cannot be conveniently accommodated (e.g., **16a**–**b** and **16d**–**h**). The phenoxybenzene moiety, as in **16c**, adds supplementary interactions with the backbone amidic hydrogens of Gly382 and Ala383 that can explain the higher affinity of this compound for the 5-$HT_{1A}$ receptor. Other derivatives, such as **16e** and **16g**, loose these supplementary interactions due to the larger size of substituents at the 6-position. The 4-methoxyphenyl group of **16n** interacts with the amidic hydrogen of the Gln97 side chain and not with the previous two aminoacids, due to a different binding mode induced by steric hindrance of the 2-methoxyphenyl group at the 1-position. The 2-furanyl ring of **5a** is not large enough to reach the pocket defined by Gly382 and Ala383; however, the affinity of this compound can be related to the H-bond between the furan oxygen and amidic group of the Asn386 side chain.

With a view to developing predictive relationships for the $\alpha_1$-AR affinity values, confirming the reliability of the obtained complexes, the computed scoring functions and a set of ligand-based descriptors (e.g., accessible surface, polar surface, apolar surface, volume, molecular weight and number of rotors) were exploited to derive correlative equations applying a step-wise linear regression approach. In Figure 5 and Eq. 1 is reported the best obtained correlation which includes only two independent variables to guarantee its statistical robustness. More in detail, the negative coefficient for $MLP_{InS}$ emphasizes the key role played by apolar contacts in complex stabilization as confirmed by the rich set of hydrophobic interactions stabilized by both phenyl rings bound to piperazine and pyrrolo-pyrimidine-2,4-dione isomers. This does not mean that ionic contacts are not relevant in ligand interaction but that they are constantly conserved in all considered complexes as seen for the ion-pair with Asp106. On the contrary, the negative coefficient of the molecular weight underlines that the binding pocket is unable to accommodate bulky ligands and probably it indirectly encodes for the detrimental interactions induced by the large aromatic systems bound to the pyrrolo-pyrimidine-2,4-dione groups. To avoid multicollinearity between independent variables, the Variance Inflation Factor (VIF) was calculated. The obtained value (1.3) is significantly less than 5, thus to indicate that there is no multicollinearity between the two independent variables. The high $R^2$ value and the presence of the $MLP_{InS}$ docking score in the correlative equation shows the good quality of the ligand–receptor complexes. Finally, the presence of $MLP_{InS}$ in Eq. 1 confirms the reliability of such a scoring function to take in account the often disregarded apolar contacts and finds useful applications to graphically evaluate the hydrophilic/hydrophobic complementary between ligand and receptor (as seen in Fig. 4 for compounds **16a** and **10e**).

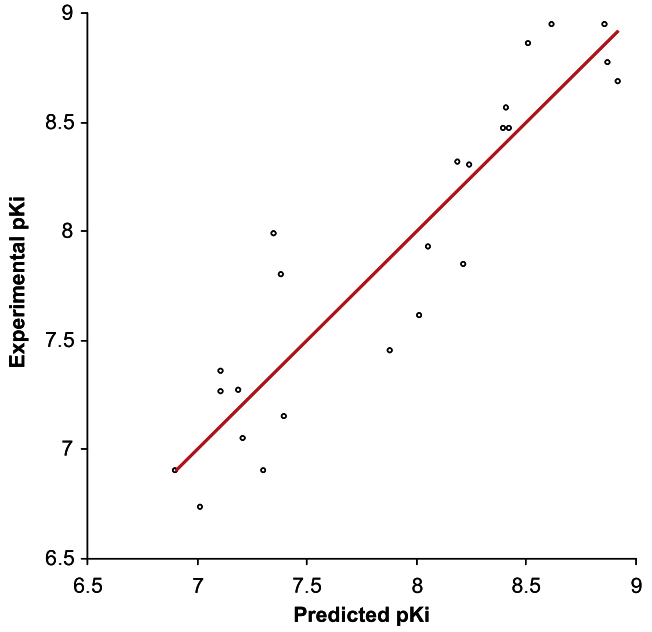



**Figure 5.** Correlation between the experimental and the predicted p$K_i$ values.

$$pK_i = 10.5(\pm 0.72) - 0.386(\pm 6.33 \times 10^{-2})MLP_{InS} - 6.302 \times 10^{-3}(\pm 1.31 \times 10^{-3})MW \quad (1)$$

$n = 22$; $R^2 = 0.85$; SE = 0.296; $F = 57.56$; $p < 0.0001$.

## 5. Conclusions

Three novel series of derivatives characterized by a 1*H*-pyrrolo-pyrimidine-2,4-dione core were synthesized and tested in radioligand binding assays to evaluate their affinity and selectivity for the $\alpha_1$-ARs with respect to 5-$HT_{1A}$ receptors. Generally, the majority of the tested compounds showed a good profile in term affinity and selectivity. In particular, compounds 3-[2-[4-(2-chlorophenyl)piperazin-1-yl]ethyl]-7-phenyl-1,5-dihydropyrrolo[3,2-*d*]pyrimidine-2,4-dione (**10b**) and 3-[2-[4-(2-chlorophenyl)piperazin-1-yl]ethyl]-7-(2-chlorophenyl)-1,5-dihydropyrrolo[3,2-*d*]pyrimidine-2,4-dione (**10c**) exhibited the highest affinity values for $\alpha_1$-ARs ($K_i$ = 1.4 nM each of them). With respect to selectivity, 3-[2-[4-(2-chlorophenyl)piperazin-1-yl]ethyl]-7-(4-chlorophenyl)-1,5-dihydropyrrolo[3,2-*d*]pyrimidine-2,4-dione (**10e**) was endowed with the best profile possessing $K_i$ = 2.71 nM at $\alpha_1$-ARs and $K_i$ >10,000 at 5-$HT_{1A}$ receptors; these results underline that the presence of a 4-chlorophenyl ring as 'second side region' and a 2-chloro substitution on the phenyl ring of the 'side pharmacophoric portion' are crucial to address high affinity and selectivity to $\alpha_1$-ARs. Molecular docking studies were performed on human $\alpha_1$-ARs and human 5-$HT_{1A}$ receptors in order to rationalize the observed experimental affinity and selectivity. These computational studies helped to clarify molecular requirements for the design of high-selective $\alpha_1$-adrenergic ligands and suggested that large aromatic substituents do not participate to the stabilization of the complex with the $\alpha_1$-receptor, for example, **16a** versus **10e**.

## 6. Experimental

### 6.1. Chemistry

Melting points were determined in a Gallenkamp apparatus with a digital thermometer MFB-595 in glass capillary tubes and

are uncorrected. Infrared spectra were recorded on a Perkin–Elmer FT IR 1600 spectrometer in KBr disks. Elemental analyses for C, H, and N were within ±0.4% of theoretical values and were performed on a Carlo Erba Elemental Analyzer Mod. 1108 apparatus. $^1$H NMR spectra were recorded at 200 MHz on a Varian Inova Unity 200 spectrometer in DMSO-$d_6$ solution. Chemical shifts are given in $\delta$ values (ppm), using tetramethylsilane as the internal standard; coupling constants ($J$) are given in hertz (Hz). Signal multiplicities are characterized as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad signal). All the synthesized compounds were tested for purity on TLC (aluminium sheet coated with silica gel F254, Merck) and visualized by UV ($\lambda$ = 254 and 366 nm). For microwave synthesis it was used a Cem Discover BenchMate apparatus. All chemicals and solvents were of reagent grade and were purchased from commercial vendors.

#### 6.1.1. 6-(2-Furanyl)-3-[2-[4-(2-methoxyphenyl)piperazin-1-yl]-ethyl]-1,5-dihydropyrrolo[3,2-*d*]pyrimidine-2,4-dione (5a)

A suspension of 3-[[[(2-chloroethyl)amino]carbonyl]amino]-5-(2-furanyl)-1*H*-pyrrole-2-carboxylic acid ethyl ester (**4a**) (0.450 g, 1.38 mmol), 1-(2-methoxyphenyl)piperazine (0.398 g, 2.07 mmol), $NaHCO_3$ (0.464 g, 5.52 mmol), and a catalytic amount of NaI in 3 mL of 2-propanol was refluxed for 4 days. After this period, the obtained mixture was concentrated, the residue was diluted with water (50 mL) and extracted with $CH_2Cl_2$ (3 × 50 mL). The combined organic layers were dried over $Na_2SO_4$, filtered, and evaporated. The obtained crude material was refluxed in methanolic KOH (1 N, 1.5 mL) for 2 h. The reaction mixture was then allowed to cool at 22 °C, acidified (pH 4) with glacial AcOH, and neutralized with saturated $NaHCO_3$. A light grey precipitate was isolated, washed with water and EtOH, and finally recrystallized from DMF/water (2:1) to afford 0.532 g of the desired product (**5a**) as solid (36%): mp 299–300 °C; IR (KBr, selected lines) cm$^{-1}$ 3174, 2947, 2820, 1708, 1636, 1587, 1500, 1439, 1250, 1029, 764; $^1$H NMR (DMSO-$d_6$) $\delta$ 12.28 (br s, 1H, NH which exchanges with $D_2O$), 11.13 (br s, 1H, NH which exchanges with $D_2O$), 7.76–7.74 (m, 1H, furane), 7.07–6.84 (m, 4H + 1H, aromatic + furane), 6.64–6.58 (m, 1H, furane), 6.09 (s, 1H, pyrrole), 4.02 (t, $J$ = 6.6 Hz, 2H, CONC$H_2$CH$_2$N), 3.77 (s, 3H, $CH_3$), 3.00–2.86 (m, 4H, $NCH_2$), 2.61–2.48 (m, 4H + 2H, $NCH_2$ + CONCH$_2$C$H_2$N). Anal. ($C_{23}H_{25}N_5O_4$) C, H, N.

By use of this procedure, the subsequent compounds were obtained.

#### 6.1.2. 3-[2-[4-(2-Chlorophenyl)piperazin-1-yl]ethyl]-6-(2-furanyl)-1,5-dihydropyrrolo[3,2-*d*]pyrimidine-2,4-dione (5b)

Starting from derivative **4a** and 1-(2-chlorophenyl)piperazine hydrochloride, the title compound (**5b**) was obtained as solid (40%): mp 301–302 °C; IR (KBr, selected lines) cm$^{-1}$ 3169, 2936, 1705, 1635, 1590, 1498, 1449, 1267, 1029, 769; $^1$H NMR (DMSO-$d_6$) $\delta$ 12.35 (br s, 1H, NH which exchanges with $D_2O$), 11.17 (br s, 1H, NH which exchanges with $D_2O$), 7.77–7.73 (m, 1H, furane), 7.43–6.95 (m, 4H + 1H, aromatic + furane), 6.63–6.58 (m, 1H, furane), 6.09 (s, 1H, pyrrole), 4.03 (t, $J$ = 6.6 Hz, 2H, CONC$H_2$CH$_2$N), 3.01–2.88 (m, 4H, $NCH_2$), 2.75–2.54 (m, 4H + 2H, $NCH_2$ + CONCH$_2$C$H_2$N). Anal. ($C_{22}H_{22}ClN_5O_3$) C, H, N.

#### 6.1.3. 6-(1,1-Dimethylethyl)-3-[2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl]-1,5-dihydropyrrolo[3,2-*d*]pyrimidine-2,4-dione (5c)

Starting from derivative **4b** and 1-(2-methoxyphenyl)piperazine, the title compound (**5c**) was obtained as solid (53%): mp 260–262 °C; IR (KBr, selected lines) cm$^{-1}$ 2998, 1699, 1635, 1584, 1449, 1239, 1014, 863, 749; $^1$H NMR (DMSO-$d_6$) $\delta$ 12.53 (br s, 1H, NH which exchanges with $D_2O$), 11.00 (br s, 1H, NH which exchanges with $D_2O$), 7.00–6.81 (m, 4H, aromatic), 6.09 (d, $^4J$ = 2.0 Hz, 1H, pyrrole), 3.99 (t, $J$ = 6.6 Hz, 2H, CONC$H_2$CH$_2$N), 3.76 (s, 3H, $CH_3$), 2.99–2.86 (m, 4H, $NCH_2$), 2.60–2.49 (m, 4H + 2H, $NCH_2$ + CONCH$_2$C$H_2$N), 1.26 (s, 9H, C(CH$_3$)$_3$). Anal. ($C_{23}H_{31}N_5O_3$) C, H, N.

#### 6.1.4. 3-[2-[4-(2-Chlorophenyl)piperazin-1-yl]ethyl]-6-(1,1-dimethylethyl)-1,5-dihydropyrrolo[3,2-*d*]pyrimidine-2,4-dione (5d)

Starting from derivative **4b** and 1-(2-chlorophenyl)piperazine hydrochloride, the title compound (**5d**) was obtained as solid (58%): mp 286–287 °C; IR (KBr, selected lines) cm$^{-1}$ 3000, 1708, 1640, 1590, 1246, 1014, 751; $^1$H NMR (DMSO-$d_6$) $\delta$ 11.54 (br s, 1H, NH which exchanges with $D_2O$), 11.02 (br s, 1H, NH which exchanges with $D_2O$), 7.43–6.95 (m, 4H, aromatic), 5.60 (s, 1H, pyrrole), 4.01 (t, $J$ = 6.6 Hz, 2H, CONC$H_2$CH$_2$N), 2.99–2.90 (m, 4H, $NCH_2$), 2.65–2.52 (m, 4H + 2H, $NCH_2$ + CONCH$_2$C$H_2$N), 1.27 (s, 9H, C(CH$_3$)$_3$). Anal. ($C_{22}H_{28}ClN_5O_2$) C, H, N.

#### 6.1.5. 3-[[[(2-Chloroethyl)amino]carbonyl]amino]-5-(2-furanyl)-1*H*-pyrrole-2-carboxylic acid ethyl ester (4a)

A suspension of 3-amino-5-(2-furanyl)-1*H*-pyrrole-2-carboxylic acid ethyl ester (**3a**) (1.00 g, 4.54 mmol)[23] and 2-chloroethyl isocyanate (0.581 mL, 6.81 mmol) in 10 mL of toluene was stirred for 16 h at 22 °C. The reaction mixture was then filtered, and the obtained solid was washed several times with EtOH to give 1.08 g of the title compound as a pure solid (73%): mp 166–168 °C; IR (KBr, selected lines) cm$^{-1}$ 3123, 1609, 1453, 1346, 1260, 1110, 762, 617; $^1$H NMR (DMSO-$d_6$) $\delta$ 11.58 (br s, 1H, NH which exchanges with $D_2O$), 8.51 (br s, 1H, N*H*CONHCH$_2$ which exchanges with $D_2O$), 7.74–7.66 (m, 1H + 1H, furane + NHCON*H*CH$_2$ which exchanges with $D_2O$), 7.02–6.98 (m, 1H + 1H, furane + pyrrole), 6.59–6.54 (m, 1H, furane), 4.32 (q, $J$ = 7.0 Hz, 2H, OC$H_2$CH$_3$), 3.66 (t, $J$ = 6.0 Hz, 2H, $CH_2Cl$), 3.46–3.40 (m, 2H, NHC$H_2$), 1.33 (t, $J$ = 7.0 Hz, 3H, OCH$_2$C$H_3$). Anal. ($C_{14}H_{16}ClN_3O_4$) C, H, N.

By use of this procedure, the subsequent compound was obtained.

#### 6.1.6. 3-[[[(2-Chloroethyl)amino]carbonyl]amino]-5-(1,1-dimethylethyl)-1*H*-pyrrole-2-carboxylic acid ethyl ester (4b)

Starting from 3-amino-5-(1,1-dimethylethyl)-1*H*-pyrrole-2-carboxylic acid ethyl ester (**3b**),[23] the title compound was obtained as a pure solid (68%): mp 150–152 °C; IR (KBr, selected lines) cm$^{-1}$ 3106, 2933, 2818, 1705, 1633, 1588, 1499, 1443, 1239, 1126, 1029, 759; $^1$H NMR (DMSO-$d_6$) $\delta$ 10.69 (br s, 1H, NH which exchanges with $D_2O$), 8.42 (br s, 1H, N*H*CONHCH$_2$ which exchanges with $D_2O$), 7.64–7.58 (m, 1H, NHCON*H*CH$_2$ which exchanges with $D_2O$), 6.53 (d, $^4J$ = 2.8 Hz, 1H, pyrrole), 4.27 (q, $J$ = 7.0 Hz, 2H, OC$H_2$CH$_3$), 3.63 (t, $J$ = 6.0 Hz, 2H, $CH_2Cl$), 3.43–3.37 (m, 2H, NHC$H_2$), 1.31 (t, $J$ = 7.0 Hz, 3H, OCH$_2$C$H_3$), 1.24 (s, 9H, C(CH$_3$)$_3$). Anal. ($C_{14}H_{22}ClN_3O_3$) C, H, N.

#### 6.1.7. 3-[2-[4-(2-Methoxyphenyl)piperazin-1-yl]ethyl]-7-phenyl-1,5-dihydropyrrolo[3,2-*d*]pyrimidine-2,4-dione (10a)

A suspension of 3-[[[(2-chloroethyl)amino]carbonyl]amino]-4-phenyl-1*H*-pyrrole-2-carboxylic acid ethyl ester (**9a**) (0.285 g, 0.85 mmol), 1-(2-methoxyphenyl)piperazine (0.245 g, 1.27 mmol), $NaHCO_3$ (0.286 g, 3.40 mmol), and a catalytic amount of NaI in 2 mL of THF was refluxed for 4 days. After this period, the obtained mixture was concentrated, the residue was diluted with water (50 mL) and extracted with $CH_2Cl_2$ (3 × 50 mL). The combined organic layers were dried over $Na_2SO_4$, filtered, and evaporated. The obtained crude material was refluxed in methanolic KOH (1 N, 1.5 mL) for 2 h. The reaction mixture was then allowed to cool at 22 °C, acidified (pH 4) with glacial AcOH, and neutralized with saturated $NaHCO_3$ solution. The obtained suspension was filtered and the isolated crude material was purified by column chromatography (Silica Gel 60, 230–400 mesh, Merck) using dichloromethane/methanol (9:1) as eluant, to afford 0.144 g of

the title compound (**10a**) as a white powder (38%): mp 274–276 °C; IR (KBr, selected lines) $cm^{-1}$ 3176, 2943, 2818, 1704, 1632, 1584, 1501, 1444, 1238, 1122, 1029, 925, 759; $^1H$ NMR (DMSO-$d_6$) $\delta$ 12.22 (br s, 1H, NH which exchanges with $D_2O$), 10.99 (br s, 1H, NH which exchanges with $D_2O$), 7.56–7.23 (m, 5H + 1H, aromatic + pyrrole), 6.93–6.84 (m, 4H, aromatic), 4.05 (t, $J$ = 6.6 Hz, 2H, CONC$H_2$CH$_2$N), 3.77 (s, 3H, $CH_3$), 3.08–2.85 (m, 4H, $NCH_2$), 2.75–2.40 (m, 4H + 2H, $NCH_2$ + CONCH$_2$C$H_2$N). Anal. ($C_{25}H_{27}N_5O_3$) C, H, N.

Using this procedure the subsequent compounds were obtained.

#### 6.1.8. 3-[2-[4-(2-Chlorophenyl)piperazin-1-yl]ethyl]-7-phenyl-1,5-dihydropyrrolo[3,2-*d*]pyrimidine-2,4-dione (10b)

Starting from derivative **9a** and 1-(2-chlorophenyl)piperazine hydrochloride, the title compound (**10b**) was obtained as a pure product (32%): mp 297–299 °C; $^1H$ NMR (DMSO-$d_6$) $\delta$ 12.21 (br s, 1H, NH which exchanges with $D_2O$), 11.00 (br s, 1H, NH which exchanges with $D_2O$), 7.57–6.97 (m, 9H + 1H, aromatic + pyrrole), 4.05 (t, $J$ = 6.6 Hz, 2H, CONC$H_2$CH$_2$N), 3.07–2.88 (m, 4H, $NCH_2$), 2.73–2.45 (m, 4H + 2H, $NCH_2$ + CONCH$_2$C$H_2$N). Anal. ($C_{24}H_{24}ClN_5O_2$) C, H, N.

#### 6.1.9. 3-[2-[4-(2-Chlorophenyl)piperazin-1-yl]ethyl]-7-(2-chlorophenyl)-1,5-dihydropyrrolo[3,2-*d*]pyrimidine-2,4-dione (10c)

Starting from derivative **9b** and 1-(2-chlorophenyl)piperazine hydrochloride, the title compound (**10c**) was obtained as a pure product (29%) mp 266–268 °C; $^1H$ NMR (DMSO-$d_6$) $\delta$ 12.22 (br s, 1H, NH which exchanges with $D_2O$), 10.99 (br s, 1H, NH which exchanges with $D_2O$), 7.59–6.85 (m, 9H + 1H, aromatic + pyrrole), 4.04 (t, $J$ = 6.6 Hz, 2H, CONC$H_2$CH$_2$N), 3.09–2.87 (m, 4H, $NCH_2$), 2.75–2.43 (m, 4H + 2H, $NCH_2$ + CONCH$_2$C$H_2$N). Anal. ($C_{24}H_{23}Cl_2N_5O_2$) C, H, N.

#### 6.1.10. 7-(4-Chlorophenyl)-3-[2-[4-(2-methoxyphenyl)piperazin-1-yl]ethyl]-1,5-dihydropyrrolo[3,2-*d*]pyrimidine-2,4-dione (10d)

Starting from derivative **9c** and 1-(2-methoxyphenyl)piperazine, the title compound (**10d**) was obtained as a pure product (35%): mp 282–284 °C; $^1H$ NMR (DMSO-$d_6$) $\delta$ 12.29 (br s, 1H, NH which exchanges with $D_2O$), 11.05 (br s, 1H, NH which exchanges with $D_2O$), 7.56–7.30 (m, 4H + 1H, aromatic + pyrrole), 7.01–6.79 (m, 4H, aromatic), 4.05 (t, $J$ = 6.6 Hz, 2H, CONC$H_2$CH$_2$N), 3.77 (s, 3H, $CH_3$), 3.15–2.83 (m, 4H, $NCH_2$), 2.77–2.39 (m, 4H + 2H, $NCH_2$ + CONCH$_2$C$H_2$N). Anal. ($C_{25}H_{26}ClN_5O_3$) C, H, N.

#### 6.1.11. 3-[2-[4-(2-Chlorophenyl)piperazin-1-yl]ethyl]-7-(4-chlorophenyl)-1,5-dihydropyrrolo[3,2-*d*]pyrimidine-2,4-dione (10e)

Starting from derivative **9c** and 1-(2-chlorophenyl)piperazine hydrochloride, the title compound (**10e**) was obtained as a pure product (39%): mp 298–300 °C; IR (KBr, selected lines) $cm^{-1}$ 3158, 2930, 1699, 1637, 1595, 1504, 1383, 1257, 1151, 763; $^1H$ NMR (DMSO-$d_6$) $\delta$ 12.22 (br s, 1H, NH which exchanges with $D_2O$), 10.99 (br s, 1H, NH which exchanges with $D_2O$), 7.58–6.97 (m, 8H + 1H, aromatic + pyrrole), 4.06 (t, $J$ = 6.6 Hz, 2H, CONC$H_2$CH$_2$N), 2.97–2.71 (m, 4H, $NCH_2$), 2.66–2.48 (m, 4H + 2H, $NCH_2$ + CONCH$_2$C$H_2$N). Anal. ($C_{24}H_{23}Cl_2N_5O_2$) C, H, N.

#### 6.1.12. 3-[2-[4-(2-Chlorophenyl)piperazin-1-yl]ethyl]-7-(4-methylphenyl)-1,5-dihydropyrrolo[3,2-*d*]pyrimidine-2,4-dione (10f)

Starting from derivative **9d** and 1-(2-chlorophenyl)piperazine hydrochloride, the title compound (**10f**) was obtained as a pure product (44%): mp 294–296 °C; $^1H$ NMR (DMSO-$d_6$) $\delta$ 12.17 (br s, 1H, NH which exchanges with $D_2O$), 10.93 (br s, 1H, NH which exchanges with $D_2O$), 7.58–6.95 (m, 8H + 1H, aromatic + pyrrole), 4.05 (t, $J$ = 6.6 Hz, 2H, CONC$H_2$CH$_2$N), 3.08–285 (m, 4H, $NCH_2$), 2.77–2.40 (m, 4H + 2H, $NCH_2$ + CONCH$_2$C$H_2$N), 2.31 (s, 3H, $CH_3$). Anal. ($C_{25}H_{26}ClN_5O_2$) C, H, N.

#### 6.1.13. 3-[[[(2-Chloroethyl)amino]carbonyl]amino]-4-phenyl-1*H*-pyrrole-2-carboxylic acid ethyl ester (9a)

A suspension of 3-amino-4-phenyl-1*H*-pyrrole-2-carboxylic acid ethyl ester (**8a**) (0.714 g, 3.10 mmol)[26] and 2-chloroethyl isocyanate (0.397 mL, 4.65 mmol) in 7 mL of toluene was stirred for 6 h at reflux. The reaction mixture was then evaporated in vacuo. The obtained crude material was purified by column chromatography (Silica Gel 60, 230–400 mesh, Merck) using cyclohexane/EtOAc (1:1) as eluant, to afford the title compound (**9a**) as pure product (0.635 g, 61%): mp 180–182 °C; IR (KBr, selected lines) $cm^{-1}$ 3100, 2962, 1666, 1612, 1528, 1407, 1360, 767, 698; $^1H$ NMR (DMSO-$d_6$) $\delta$ 11.85 (br s, 1H, NH which exchanges with $D_2O$), 7.64 (br s, 1H, N*H*CONHCH$_2$ which exchanges with $D_2O$), 7.54–7.12 (m, 5H + 1H, aromatic + pyrrole), 6.47 (t, $J$ = 5.8 Hz, 1H, NHCON*H*CH$_2$ which exchanges with $D_2O$), 4.21 (q, $J$ = 7.0 Hz, 2H, OC$H_2$CH$_3$), 3.52 (t, $J$ = 6.0 Hz, 2H, CH$_2$Cl), 3.33–3.24 (m, 2H, NHC$H_2$), 1.27 (t, $J$ = 7.0 Hz, 3H, OCH$_2$C$H_3$). Anal. ($C_{16}H_{18}ClN_3O_3$) C, H, N.

Using this procedure the subsequent compounds were obtained.

#### 6.1.14. 3-[[[(2-Chloroethyl)amino]carbonyl]amino]-4-(2-chlorophenyl)-1*H*-pyrrole-2-carboxylic acid ethyl ester (9b)

Starting from derivative **8b**, the title compound (**9b**) was obtained as a pure product (66%): mp 182–184 °C; $^1H$ NMR (DMSO-$d_6$) $\delta$ 11.86 (br s, 1H, NH which exchanges with $D_2O$), 7.63 (br s, 1H, N*H*CONHCH$_2$ which exchanges with $D_2O$), 7.52–7.19 (m, 4H, aromatic), 7.05 (d, $J$ = 3.4 Hz, 1H, pyrrole), 6.58 (t, $J$ = 5.8 Hz, 1H, NHCON*H*CH$_2$ which exchanges with $D_2O$), 4.23 (q, $J$ = 7.0 Hz, 2H, OC$H_2$CH$_3$), 3.46 (t, $J$ = 6.0 Hz, 2H, CH$_2$Cl), 3.35–3.15 (m, 2H, NHC$H_2$), 1.28 (t, $J$ = 7.0 Hz, 3H, OCH$_2$C$H_3$). Anal. ($C_{16}H_{17}Cl_2N_3O_3$) C, H, N.

#### 6.1.15. 3-[[[(2-Chloroethyl)amino]carbonyl]amino]-4-(4-chlorophenyl)-1*H*-pyrrole-2-carboxylic acid ethyl ester (9c)

Starting from derivative **8c**, the title compound (**9c**) was obtained as a pure product (70%): mp 214–216 °C; IR (KBr, selected lines) $cm^{-1}$ 3270, 1678, 1642, 1567, 1423, 1253, 1146, 1015, 766, 653; $^1H$ NMR (DMSO-$d_6$) $\delta$ 11.80 (br s, 1H, NH which exchanges with $D_2O$), 7.68 (br s, 1H, N*H*CONHCH$_2$ which exchanges with $D_2O$), 7.54–7.48 (m, 2H, aromatic), 7.38–7.22 (m, 2H + 1H, aromatic + pyrrole), 6.55 (t, $J$ = 5.8 Hz, 1H, NHCON*H*CH$_2$ which exchanges with $D_2O$), 4.22 (q, $J$ = 7.0 Hz, 2H, OC$H_2$CH$_3$), 3.54 (t, $J$ = 6.0 Hz, 2H, CH$_2$Cl), 3.33–3.27 (m, 2H, NHC$H_2$), 1.28 (t, $J$ = 7.0 Hz, 3H, OCH$_2$C$H_3$). Anal. ($C_{16}H_{17}Cl_2N_3O_3$) C, H, N.

#### 6.1.16. 3-[[[(2-Chloroethyl)amino]carbonyl]amino]-4-(4-methylphenyl)-1*H*-pyrrole-2-carboxylic acid ethyl ester (9d)

Starting from derivative **8d**, the title compound (**9d**) was obtained as a pure product (65%): mp 204–206 °C; $^1H$ NMR (DMSO-$d_6$) $\delta$ 11.79 (br s, 1H, NH which exchanges with $D_2O$), 7.59 (br s, 1H, N*H*CONHCH$_2$ which exchanges with $D_2O$), 7.47–7.29 (m, 2H, aromatic), 7.22–7.00 (m, 2H + 1H, aromatic + pyrrole), 6.42 (t, $J$ = 5.8 Hz, 1H, NHCON*H*CH$_2$ which exchanges with $D_2O$), 4.20 (q, $J$ = 7.0 Hz, 2H, OC$H_2$CH$_3$), 3.53 (t, $J$ = 6.0 Hz, 2H, CH$_2$Cl), 3.40–3.22 (m, 2H, NHC$H_2$), 2.27 (s, 3H, $CH_3$), 1.27 (t, $J$ = 7.0 Hz, 3H, OCH$_2$C$H_3$). Anal. ($C_{17}H_{20}ClN_3O_3$) C, H, N.

#### 6.1.17. 3-Amino-4-(2-chlorophenyl)-1*H*-pyrrole-2-carboxylic acid ethyl ester (8b)

Sodium (0.430 g, 18.7 mmol) was dissolved in 20 mL of absolute ethanol and then *N*-[2-cyano-2-(2-chlorophenyl)ethenyl]glycine

ethyl ester (**7b**) (4.0 g, 17.3 mmol) was added. The reaction mixture was stirred at 22 °C for 48 h and then poured into 300 mL of water. The suspension was filtered and the solid was dissolved in $CH_2Cl_2$. The obtained organic phase was extracted with saturated sodium chloride solution, dried over over $Na_2SO_4$, filtered, and evaporated. The isolated crude material was purified by column chromatography (Silica Gel 60, 230–400 mesh, Merck) using EtOAc/cyclohexane (3:7) as eluant. The title compound (**8b**) was obtained as a pure product (2.8 g, 60%): mp 100–102 °C; IR (KBr, selected lines) $cm^{-1}$ 3143, 2985, 1695, 1685, 1519, 1411, 1297, 1140, 1033, 765; $^1H$ NMR (DMSO-$d_6$) $\delta$ 11.05 (br s, 1H, NH which exchanges with $D_2O$), 7.55–7.21 (m, 4H, aromatic) 6.90 (d, $^4J$ = 3.8 Hz, 1H, pyrrole), 4.85 (br s, 2H, $NH_2$ which exchanges with $D_2O$), 4.23 (q, $J$ = 7.0 Hz, 2H, $OCH_2CH_3$), 1.29 (t, $J$ = 7.0 Hz, 3H, $OCH_2CH_3$). Anal. ($C_{13}H_{13}ClN_2O_2$) C, H, N.

### 6.1.18. 3-Amino-4-(4-chlorophenyl)-1*H*-pyrrole-2-carboxylic acid ethyl ester (8c)[27]

Starting from derivative **7c**, the title compound (**8c**) was obtained as a pure product (66%): mp 117–119 °C; $^1H$ NMR (DMSO-$d_6$) $\delta$ 11.06 (br s, 1H, NH which exchanges with $D_2O$), 7.50–7.21 (m, 4H, aromatic), 6.91 (d, $^4J$ = 3.8 Hz, 1H, pyrrole), 4.86 (br s, 2H, $NH_2$ which exchanges with $D_2O$), 4.22 (q, $J$ = 7.0 Hz, 2H, $OCH_2CH_3$), 1.28 (t, $J$ = 7.0 Hz, 3H, $OCH_2CH_3$). Anal. ($C_{13}H_{13}ClN_2O_2$) C, H, N.

### 6.1.19. 3-Amino-4-(4-methylphenyl)-1*H*-pyrrole-2-carboxylic acid ethyl ester (8d)

Starting from derivative **7d**, the title compound (**8d**) was obtained as a pure product (78%): mp 98–100 °C; IR (KBr, selected lines) $cm^{-1}$ 3155, 2982, 1676, 1587, 1488, 1441, 1374, 1301, 1133, 827, 794; $^1H$ NMR (DMSO-$d_6$) $\delta$ 10.96 (br s, 1H, NH which exchanges with $D_2O$), 7.45–7.30 (m, 2H, aromatic), 7.20–7.09 (m, 2H, aromatic), 6.98 (d, $^4J$ = 3.8 Hz, 1H, pyrrole), 5.00 (br s, 2H, $NH_2$ which exchanges with $D_2O$), 4.22 (q, $J$ = 7.0 Hz, 2H, $OCH_2CH_3$), 2.29 (s, 3H, $CH_3$), 1.28 (t, $J$ = 7.0 Hz, 3H, $OCH_2CH_3$). Anal. ($C_{14}H_{16}N_2O_2$) C, H, N.

### 6.1.20. *N*-[2-Cyano-2-(2-chlorophenyl)ethenyl]glycine ethyl ester (7b)

Glycine ethyl ester hydrochloride (2.5 g, 17.9 mmol) was suspended in 200 mL of toluene and TEA (2.5 mL, 17.9 mmol), α-formyl-2-chlorobenzeneacetonitrile (**6b**) (3.2 g, 17.9 mmol),[24] and 4-methyl-benzenesulfonic acid (0.05 g, 0.263 mmol) were added. Stirring was carried out under reflux using a water separator, for 1.5 h. The reaction mixture was cooled to room temperature, filtered and evaporated. The isolated crude material was purified by column chromatography (Silica Gel 60, 230–400 mesh, Merck) using EtOAc/cyclohexane (1:1) as eluant, to afford 3.8 g of the title compound (**7b**) (80%): $^1H$ NMR (DMSO-$d_6$) $\delta$ 7.81 (d, $J$ = 13.2 Hz, 1H, C*H*NH), 7.61–7.54 (m, 1H, NH which exchanges with $D_2O$), 7.40–7.22 (m, 4H, aromatic), 4.15 (q, $J$ = 7.0 Hz, 2H, $OCH_2CH_3$), 4.05 (d, $J$ = 5.6 Hz, 2H, $NHCH_2$), 1.22 (t, $J$ = 7.0 Hz, 3H, $OCH_2CH_3$). Anal. ($C_{13}H_{13}ClN_2O_2$) C, H, N. Using this procedure the subsequent compounds were obtained.

### 6.1.21. *N*-[2-Cyano-2-(4-chlorophenyl)ethenyl]glycine ethyl ester (7c)

Starting from derivative **6c**, the title compound (**7c**) was obtained as a pure product (87%): mp 82–84 °C; $^1H$ NMR (DMSO-$d_6$) $\delta$ 7.80 (d, $J$ = 13.2 Hz, 1H, C*H*NH), 7.60–7.53 (m, 1H, NH which exchanges with $D_2O$), 7.39–7.23 (m, 4H, aromatic), 4.15 (q, $J$ = 7.0 Hz, 2H, $OCH_2CH_3$), 4.06 (d, $J$ = 5.6 Hz, 2H, $NHCH_2$), 1.22 (t, $J$ = 7.0 Hz, 3H, $OCH_2CH_3$). Anal. ($C_{13}H_{13}ClN_2O_2$) C, H, N.

### 6.1.22. *N*-[2-Cyano-2-(4-methylphenyl)ethenyl]glycine ethyl ester (7d)

Starting from derivative **6d**,[14] the title compound (**7d**) was obtained as a pure product (88%): mp 88–90 °C; $^1H$ NMR (DMSO-$d_6$) $\delta$ 7.65 (d, $J$ = 13.2 Hz, 1H, C*H*NH), 7.39–7.22 (m, 1H, NH which exchanges with $D_2O$), 7.20–7.05 (m, 4H, aromatic), 4.16 (q, $J$ = 7.0 Hz, 2H, $OCH_2CH_3$), 4.04 (d, $J$ = 5.6 Hz, 2H, $NHCH_2$), 2.25 (s, 3H, $CH_3$), 1.22 (t, $J$ = 7.0 Hz, 3H, $OCH_2CH_3$). Anal. ($C_{14}H_{16}N_2O_2$) C, H, N.

### 6.1.23. 6-[4-(1,1'-Biphenyl)]-3-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-1*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione (16a)

A suspension of 5-[4-(1,1'-biphenyl)]-2-[[[[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]amino]carbonyl]amino]-1*H*-pyrrole-3-carboxylic acid ethyl ester (**15a**) (0.227 g, 0.40 mmol) in methanolic KOH (1 N, 1.5 mL) was microwave irradiated at 120 °C, for 15 min, (200 W, 150 psi) in a sealed tube. The reaction mixture was then allowed to cool at 22 °C, acidified (pH 4) with glacial AcOH, and neutralized with saturated $NaHCO_3$. A white precipitate was isolated, washed with water and triturated in EtOAc to afford the title compound (**16a**) as a white solid (0.144 g, 69%): mp 243–245 °C; IR (KBr, selected lines) $cm^{-1}$ 2827, 1704, 1637, 1578, 1496, 1240, 1023, 759, 608; $^1H$ NMR (DMSO-$d_6$) $\delta$ 12.17 (br s, 1H + 1H, NH + NH which exchange with $D_2O$), 7.87–7.32 (m, 9H, aromatic), 6.94–6.81 (m, 4H + 1H, aromatic + pyrrole), 4.04 (t, $J$ = 6.6 Hz, 2H, $CONCH_2CH_2N$), 3.75 (s, 3H, $CH_3$), 2.96–2.90 (m, 4H, $NCH_2$), 2.65–2.50 (m, 4H + 2H, $NCH_2$ + $CONCH_2CH_2N$). Anal. ($C_{31}H_{31}N_5O_3$) C, H, N.

Using this procedure the subsequent compounds were obtained.

### 6.1.24. 6-[4-(1,1'-Biphenyl)]-3-[2-[4-(2-chlorophenyl)-1-piperazinyl]ethyl]-1*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione (16b)

Starting from derivative **15b**, the title compound (**16b**) was obtained as a white solid (69%): mp 266–268 °C; IR (KBr, selected lines) $cm^{-1}$ 2820, 1704, 1581, 1479, 1443, 1118, 1038, 836, 757, 609; $^1H$ NMR (DMSO-$d_6$) $\delta$ 11.98 (br s, 1H + 1H, NH + NH which exchange with $D_2O$), 7.90–6.91 (m, 13H, aromatic), 6.85 (s, 1H, pyrrole), 4.03 (t, $J$ = 6.6 Hz, 2H, $CONCH_2CH_2N$), 3.01–2.94 (m, 4H, $NCH_2$), 2.69–2.55 (m, 4H + 2H, $NCH_2$ + $CONCH_2CH_2N$). Anal. ($C_{30}H_{28}ClN_5O_2$) C, H, N.

### 6.1.25. 3-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-6-(4-phenoxyphenyl)-1*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione (16c)

Starting from derivative **15c**, the title compound (**16c**) was obtained as a white solid (68%): mp 275–277 °C; IR (KBr, selected lines) $cm^{-1}$ 3023, 1699, 1637, 1581, 1491, 1241, 1115, 1018, 749; $^1H$ NMR (DMSO-$d_6$) $\delta$ 12.13 (br s, 1H + 1H, NH + NH which exchange with $D_2O$), 7.77–7.64 (m, 2H, aromatic), 7.42–7.29 (m, 2H, aromatic), 7.20–6.70 (m, 9H, aromatic), 6.66 (s, 1H, pyrrole), 4.06 (t, $J$ = 6.6 Hz, 2H, $CONCH_2CH_2N$), 3.74 (s, 3H, $CH_3$), 2.93–2.87 (m, 4H, $NCH_2$), 2.64–2.45 (m, 4H + 2H, $NCH_2$ + $CONCH_2CH_2N$). Anal. ($C_{31}H_{31}N_5O_4$) C, H, N.

### 6.1.26. 3-[2-[4-(2-Chlorophenyl)-1-piperazinyl]ethyl]-6-(4-phenoxyphenyl)-1*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione (16d)

Starting from derivative **15d**, the title compound (**16d**) was obtained as a white solid (77%): mp 266–268 °C; IR (KBr, selected lines) $cm^{-1}$ 2896, 1702, 1637, 1578, 1487, 1243, 1041, 936, 753; $^1H$ NMR (DMSO-$d_6$) $\delta$ 12.01 (br s, 1H + 1H, NH + NH which exchange with $D_2O$), 7.78–7.66 (m, 2H, aromatic), 7.47–6.95 (m,

11H, aromatic), 6.71 (s, 1H, pyrrole), 4.02 (t, $J$ = 6.6 Hz, 2H, CONC$H_2$CH$_2$N), 2.98–2.90 (m, 4H, NCH$_2$), 2.65–2.44 (m, 4H + 2H, NCH$_2$ + CONCH$_2$C$H_2$N). Anal. ($C_{30}H_{28}ClN_5O_3$) C, H, N.

#### 6.1.27. 3-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-6-[4-(phenylmethoxy)phenyl]-1*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione (16e)

Starting from derivative **15e**, the title compound (**16e**) was obtained as a white solid (69%): mp >300 °C; IR (KBr, selected lines) cm$^{-1}$ 2994, 1701, 1632, 1582, 1448, 1241, 1016, 863, 747; $^1$H NMR (DMSO-$d_6$) $\delta$ 11.96 (br s, 1H + 1H, NH + NH which exchange with D$_2$O), 7.62–7.29 (m, 7H, aromatic), 7.01–6.79 (m, 6H, aromatic), 6.41 (s, 1H, pyrrole), 5.09 (s, 2H, CH$_2$O), 4.02 (t, $J$ = 6.6 Hz, 2H, CONC$H_2$CH$_2$N), 3.75 (s, 3H, CH$_3$), 3.02–2.92 (m, 4H, NCH$_2$), 2.66–2.41 (m, 4H + 2H, NCH$_2$ + CONCH$_2$C$H_2$N). Anal. ($C_{32}H_{33}N_5O_4$) C, H, N.

#### 6.1.28. 3-[2-[4-(2-Chlorophenyl)-1-piperazinyl]ethyl]-6-[4-(phenylmethoxy)phenyl]-1*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione (16f)

Starting from derivative **15f**, the title compound (**16f**) was obtained as a white solid (63%): mp >300 °C; IR (KBr, selected lines) cm$^{-1}$ 3035, 1699, 1632, 1583, 1483, 14444, 1243, 1013, 758; $^1$H NMR (DMSO-$d_6$) $\delta$ 11.78 (br s, 1H + 1H, NH + NH which exchange with D$_2$O), 7.69–7.58 (m, 2H, aromatic), 7.46–6.95 (m, 11H, aromatic), 6.62 (s, 1H, pyrrole), 5.13 (s, 2H, CH$_2$O), 4.02 (t, $J$ = 6.6 Hz, 2H, CONC$H_2$CH$_2$N), 3.04–2.93 (m, 4H, NCH$_2$), 2.65–2.47 (m, 4H + 2H, NCH$_2$ + CONCH$_2$C$H_2$N). Anal. ($C_{31}H_{30}ClN_5O_3$) C, H, N.

#### 6.1.29. 3-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-6-[4-(2-phenoxyethoxy)phenyl]-1*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione (16g)

Starting from derivative **15g**, the title compound (**16g**) was obtained as a white solid (50%): mp >300 °C; IR (KBr, selected lines) cm$^{-1}$ 2986, 1705, 1655, 1447, 1389, 1247, 1132, 1060, 976, 751, 699; $^1$H NMR (DMSO-$d_6$) $\delta$ 12.01 (br s, 1H + 1H, NH + NH which exchange with D$_2$O), 7.69–6.91 (m, 13H, aromatic), 6.42 (s, 1H, pyrrole), 4.30 (m, 2H + 2H, OCH$_2$CH$_2$O), 4.03 (t, $J$ = 6.6 Hz, 2H, CONC$H_2$CH$_2$N), 3.76 (s, 3H, CH$_3$), 3.04–2.90 (m, 4H, NCH$_2$), 2.64–2.42 (m, 4H + 2H, NCH$_2$ + CONCH$_2$C$H_2$N). Anal. ($C_{33}H_{35}N_5O_5$) C, H, N.

#### 6.1.30. 3-[2-[4-(2-Chlorophenyl)-1-piperazinyl]ethyl]-6-[4-(2-phenoxyethoxy)phenyl]-1*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione (16h)

Starting from derivative **15h**, the title compound (**16h**) was obtained as a white solid (53%): mp >300 °C; IR (KBr, selected lines) cm$^{-1}$, 3001, 1704, 1653, 1452, 1370, 1240, 1057, 936, 755, 692; $^1$H NMR (DMSO-$d_6$) $\delta$ 11.95 (br s, 1H + 1H, NH + NH which exchange with D$_2$O), 7.63–6.87 (m, 13H, aromatic), 6.39 (s, 1H, pyrrole), 4.30 (m, 2H + 2H, OCH$_2$CH$_2$O), 4.00 (t, $J$ = 6.6 Hz, 2H, CONC$H_2$CH$_2$N), 3.02–2.94 (m, 4H, NCH$_2$), 2.67–2.49 (m, 4H + 2H, NCH$_2$ + CONCH$_2$C$H_2$N). Anal. ($C_{32}H_{32}ClN_5O_4$) C, H, N.

#### 6.1.31. 3-[2-[4-(2-Chlorophenyl)-1-piperazinyl]ethyl]-6-phenyl-1-(phenylmethyl)-1*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione (16l)

Starting from derivative **15i**, the title compound (**16l**) was obtained as a white solid (78%): mp 128–130 °C; IR (KBr, selected lines) cm$^{-1}$ 2829, 1691, 1640, 1559, 1442, 1309, 1120, 1008, 934, 703; $^1$H NMR (DMSO-$d_6$) $\delta$ 11.74 (br s, 1H, NH which exchanges with D$_2$O), 7.75–7.68 (m, 2H, aromatic), 7.45–7.21 (m, 10H, aromatic), 7.11–6.99 (m, 2H, aromatic), 6.87 (s, 1H, pyrrole), 5.35 (s, 2H, NCH$_2$Ar), 4.06 (t, $J$ = 6.6 Hz, 2H, CONC$H_2$CH$_2$N), 2.91–2.86 (m, 4H, NCH$_2$), 2.62–2.52 (m, 4H + 2H, NCH$_2$ + CONCH$_2$C$H_2$N). Anal. ($C_{31}H_{30}ClN_5O_2$) C, H, N.

#### 6.1.32. 5,6-Diphenyl-3-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-1*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione (16i)

A suspension of 2-[[[(2-chloroethyl)amino]carbonyl]amino]-4,5-diphenyl-1*H*-pyrrole-3-carboxylic acid ethyl ester (**14e**) (0.329 g, 0.80 mmol), 1-(2-methoxyphenyl)piperazine (0.185 g, 0.96 mmol), NaHCO$_3$ (0.269 g, 3.2 mmol), and a catalytic amount of NaI in 2 mL of THF was refluxed for 24 h. After this period, the obtained mixture was concentrated, the residue was diluted with water (30 mL) and extracted with EtOAc (3 × 30 mL). The combined organic layers were dried over Na$_2$SO$_4$, filtered and evaporated. The mixture was dissolved in EtOAc, filtered through a pad of silica gel, concentrated and used into the next step without any further purification. The crude 4,5-diphenyl-2-[[[[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]amino]carbonyl]amino]-1*H*-pyrrole-3-carboxylic acid ethyl ester was suspended in methanolic KOH (1 N, 3.0 mL) and microwave irradiated at 120 °C, for 15 min, (200 W, 150 psi) in a sealed tube. The reaction mixture was then allowed to cool at 22 °C, acidified (pH 4) with glacial AcOH, and neutralized with saturated NaHCO$_3$. A white precipitate was isolated, washed with water and triturated in EtOAc to afford the title compound (**16i**) as a white solid (72%): mp 184–186 °C; IR (KBr, selected lines) cm$^{-1}$ 2943, 2821, 1701, 1651, 1593, 1487, 1447, 1302, 1238, 1110, 697; $^1$H NMR (DMSO-$d_6$) $\delta$ 11.85 (br s, 1H + 1H, NH + NH which exchange with D$_2$O), 7.30–7.17 (m, 10H, aromatic), 6.98–6.79 (m, 4H, aromatic), 3.98 (t, $J$ = 6.6 Hz, 2H, CONC$H_2$CH$_2$N), 3.76 (s, 3H, CH$_3$), 2.97–2.89 (m, 4H, NCH$_2$), 2.62–2.51 (m, 4H + 2H, NCH$_2$ + CONCH$_2$C$H_2$N). Anal. ($C_{31}H_{31}N_5O_3$) C, H, N.

Using this procedure the subsequent compounds were obtained.

#### 6.1.33. 3-[2-[4-(2-Chlorophenyl)-1-piperazinyl]ethyl]-5,6-diphenyl-1*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione (16k)

Starting from derivative **14e**, and 1-(2-chlorophenyl)piperazine hydrochloride, the title compound (**16k**) was obtained as a white solid (81%): mp 246–248 °C; IR (KBr, selected lines) cm$^{-1}$ 2983, 2820, 1702, 1639, 1595, 1442, 1231, 1035, 760, 699; $^1$H NMR (DMSO-$d_6$) $\delta$ 11.85 (br s, 1H + 1H, NH + NH which exchange with D$_2$O), 7.45–6.98 (m, 14H, aromatic), 3.98 (t, $J$ = 6.6 Hz, 2H, CONC$H_2$CH$_2$N), 3.05–2.94 (m, 4H, NCH$_2$), 2.68–2.47 (m, 4H + 2H, NCH$_2$ + CONCH$_2$C$H_2$N). Anal. ($C_{30}H_{28}ClN_5O_2$) C, H, N.

#### 6.1.34. 3-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-6-phenyl-1-(phenylmethyl)-1*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione (16j)

Starting from derivative **14f** and 1-(2-methoxyphenyl)piperazine, the title compound (**16j**) was obtained as a white solid (76%): mp 126–127 °C; IR (KBr, selected lines) cm$^{-1}$ 3065, 2831, 1693, 1639, 1559, 1445, 1241, 1026, 755, 707; $^1$H NMR (DMSO-$d_6$) $\delta$ 11.74 (br s, 1H, NH which exchanges with D$_2$O), 7.75–7.65 (m, 2H, aromatic), 7.45–7.23 (m, 8H, aromatic), 6.95–6.82 (m, 4H + 1H, aromatic + pyrrole), 5.35 (s, 2H, NCH$_2$Ar), 4.06 (t, $J$ = 6.6 Hz, 2H, CONC$H_2$CH$_2$N), 3.76 (s, 3H, CH$_3$), 2.93–2.87 (m, 4H, NCH$_2$), 2.56–2.48 (m, 4H + 2H, NCH$_2$ + CONCH$_2$C$H_2$N). Anal. ($C_{32}H_{33}N_5O_3$) C, H, N.

#### 6.1.35. 3-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-6-(4-methoxyphenyl)-1-[(2-methoxyphenyl)methyl]-1*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione (16m)

Starting from derivative **14g** and 1-(2-methoxyphenyl)piperazine, the title compound (**16m**) was obtained as a white solid (70%): mp 219–221 °C; IR (KBr, selected lines) cm$^{-1}$ 3021, 2831, 1655, 1563, 1499, 1243, 1114, 1024, 828, 745; $^1$H NMR (DMSO-$d_6$) $\delta$ 11.56 (br s, 1H, NH which exchanges with D$_2$O), 7.71–7.55

(m, 2H, aromatic), 7.30–7.15 (m, 1H, aromatic), 7.10–6.65 (m, 9H + 1H, aromatic + pyrrole), 5.25 (s, 2H, $NCH_2Ar$), 4.04 (t, $J$ = 6.6 Hz, 2H, $CONCH_2CH_2N$), 3.87 (s, 3H, $CH_3$), 3.76 (s, 3H, $CH_3$), 3.75 (s, 3H, $CH_3$), 2.90–2.83 (m, 4H, $NCH_2$), 2.58–2.47 (m, 4H + 2H, $NCH_2$ + $CONCH_2CH_2N$). Anal. ($C_{34}H_{37}N_5O_5$) C, H, N.

#### 6.1.36. 3-[2-[4-(2-Chlorophenyl)-1-piperazinyl]ethyl]-6-(4-methoxyphenyl)-1-[(2-methoxyphenyl)methyl]-1*H*-pyrrolo[2,3-*d*]pyrimidine-2,4(3*H*,7*H*)-dione (16n)

Starting from derivative **14g** and 1-(2-chlorophenyl)piperazine hydrochloride, the title compound (**16n**) was obtained as a white solid (65%): mp 198–199 °C; IR (KBr, selected lines) $cm^{-1}$ 3106, 2929, 1692, 1653, 1562, 1500, 1252, 1122, 1031, 762; $^1H$ NMR (DMSO-$d_6$) $\delta$ 11.56 (br s, 1H, NH which exchanges with $D_2O$), 7.70–7.58 (m, 2H, aromatic), 7.43–7.17 (m, 3H, aromatic), 7.15–6.69 (m, 7H + 1H, aromatic + pyrrole), 5.25 (s, 2H, $NCH_2Ar$), 4.05 (t, $J$ = 6.6 Hz, 2H, $CONCH_2CH_2N$), 3.87 (s, 3H, $CH_3$), 3.76 (s, 3H, $CH_3$), 2.93–2.81 (m, 4H, $NCH_2$), 2.60–2.47 (m, 4H + 2H, $NCH_2$ + $CONCH_2CH_2N$). Anal. ($C_{33}H_{34}ClN_5O_4$) C, H, N.

#### 6.1.37. 5-[4-(1,1'-Biphenyl)]-2-[[[[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]amino]carbonyl]amino]-1*H*-pyrrole-3-carboxylic acid ethyl ester (15a)

A suspension of 5-[4-(1,1'-biphenyl)]-2-[[[(2-chloroethyl)-amino]carbonyl]amino]-1*H*-pyrrole-3-carboxylic acid ethyl ester (**14a**) (0.329 g, 0.80 mmol), 1-(2-methoxyphenyl)piperazine (0.185 g, 0.96 mmol), $NaHCO_3$ (0.269 g, 3.2 mmol), and a catalytic amount of NaI in 2 mL of THF was refluxed for 24 h. After this period, the obtained mixture was concentrated, the residue was diluted with water (30 mL) and extracted with EtOAc (3 × 30 mL). The combined organic layers were dried over $Na_2SO_4$, filtered and evaporated. The obtained crude material was purified by column chromatography (Silica Gel 60, 230–400 mesh, Merck) using EtOAc (100%) as eluant, to afford 0.354 g of the title compound (**15a**) as a white powder (78%): mp 176–178 °C; IR (KBr, selected lines) $cm^{-1}$ 2937, 2820, 1705, 1597, 1465, 1380, 1245, 1106, 759, 588; $^1H$ NMR (DMSO-$d_6$) $\delta$ 11.43 (br s, 1H, NH which exchanges with $D_2O$), 9.30 (br s, 1H, N*H*CONHCH$_2$ which exchanges with $D_2O$), 7.73–7.32 (m, 9H + 1H, aromatic + NHCON*H*CH$_2$ which exchanges with $D_2O$), 6.95–6.84 (m, 4H, aromatic), 6.73 (s, 1H, pyrrole), 4.24 (q, $J$ = 7.0 Hz, 2H, O*CH*$_2$CH$_3$), 3.77 (s, 3H, $CH_3$), 3.39–3.32 (m, 2H, NHC*H*$_2$), 3.10–2.95 (m, 4H, $NCH_2$), 2.65–2.49 (m, 4H + 2H, $NCH_2$ + $CONHCH_2CH_2N$), 1.30 (t, $J$ = 7.0 Hz, 3H, $OCH_2CH_3$). Anal. ($C_{33}H_{37}N_5O_4$) C, H, N.

Using this procedure the subsequent compounds were obtained.

#### 6.1.38. 5-[4-(1,1'-Biphenyl)]-2-[[[[2-[4-(2-chlorophenyl)-1-piperazinyl]ethyl]amino]carbonyl]amino]-1*H*-pyrrole-3-carboxylic acid ethyl ester (15b)

Starting from derivative **14a** and 1-(2-chlorophenyl)piperazine hydrochloride, compound **15b** was obtained as crude material. The mixture was purified by column chromatography (Silica Gel 60, 230–400 mesh, Merck) using EtOAc (100%) as eluant to afford the pure title compound (75%): mp 188–190 °C; IR (KBr, selected lines) $cm^{-1}$ 2903, 1698, 1641, 1535, 1262, 1095, 924, 764; $^1H$ NMR (DMSO-$d_6$) $\delta$ 11.43 (br s, 1H, NH which exchanges with $D_2O$), 9.29 (br s, 1H, N*H*CONHCH$_2$ which exchanges with $D_2O$), 7.78–6.99 (m, 13H + 1H, aromatic + NHCON*H*CH$_2$ which exchanges with $D_2O$), 6.72 (s, 1H, pyrrole), 4.24 (q, $J$ = 7.0 Hz, 2H, O*CH*$_2$CH$_3$), 3.38–3.30 (m, 2H, NHC*H*$_2$), 3.03–2.92 (m, 4H, $NCH_2$), 2.58–2.46 (m, 4H + 2H, $NCH_2$ + $CONHCH_2CH_2N$), 1.30 (t, $J$ = 7.0 Hz, 3H, $OCH_2CH_3$). Anal. ($C_{32}H_{34}ClN_5O_3$) C, H, N.

#### 6.1.39. 2-[[[[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-amino]carbonyl]amino]-5-(4-phenoxyphenyl)-1*H*-pyrrole-3-carboxylic acid ethyl ester (15c)

Starting from derivative **14b** and 1-(2-methoxyphenyl)piperazine, compound **15c** was obtained as crude material. The mixture was purified by column chromatography (Silica Gel, 60 230–400 mesh, Merck) using EtOAc (100%) as eluant (69%) to afford the pure title compound: mp 166–168 °C; IR (KBr, selected lines) $cm^{-1}$ 3044, 2935, 1709, 1662, 1595, 1247, 1104, 741; $^1H$ NMR (DMSO-$d_6$) $\delta$ 11.32 (br s, 1H, NH which exchanges with $D_2O$), 9.24 (br s, 1H, N*H*CONHCH$_2$ which exchanges with $D_2O$), 7.70 (br t, 1H, NHCON*H*CH$_2$ which exchanges with $D_2O$), 7.63–7.32 (m, 4H, aromatic), 7.20–6.84 (m, 9H, aromatic), 6.58 (s, 1H, pyrrole), 4.22 (q, $J$ = 7.0 Hz, 2H, O*CH*$_2$CH$_3$), 3.76 (s, 3H, $CH_3$), 3.30–3.22 (m, 2H, NHC*H*$_2$), 3.06–2.94 (m, 4H, $NCH_2$), 2.62–2.50 (m, 4H + 2H, $NCH_2$ + $CONHCH_2CH_2N$), 1.28 (t, $J$ = 7.0 Hz, 3H, $OCH_2CH_3$). Anal. ($C_{33}H_{37}N_5O_5$) C, H, N.

#### 6.1.40. 2-[[[[2-[4-(2-Chlorophenyl)-1-piperazinyl]ethyl]amino]-carbonyl]amino]-5-(4-phenoxyphenyl)-1*H*-pyrrole-3-carboxylic acid ethyl ester (15d)

Starting from derivative **14b** and 1-(2-chlorophenyl)piperazine hydrochloride, compound **15d** was obtained as crude material. The mixture was purified by column chromatography (Silica Gel 60, 230–400 mesh, Merck) using EtOAc (100%) as eluant to afford the pure title compound (77%): mp 139–141 °C; IR (KBr, selected lines) $cm^{-1}$ 3102, 2364, 1657, 1595, 1484, 1246, 1103, 754; $^1H$ NMR (DMSO-$d_6$) $\delta$ 11.32 (br s, 1H, NH which exchanges with $D_2O$), 9.24 (br s, 1H, N*H*CONHCH$_2$ which exchanges with $D_2O$), 7.70 (br t, 1H, NHCON*H*CH$_2$ which exchanges with $D_2O$), 7.62–7.53 (m, 3H, aromatic), 7.45–6.90 (m, 10H, aromatic), 6.58 (s, 1H, pyrrole), 4.22 (q, $J$ = 7.0 Hz, 2H, O*CH*$_2$CH$_3$), 3.34–3.28 (m, 2H, NHC*H*$_2$), 3.11–2.98 (m, 4H, $NCH_2$), 2.64–2.50 (m, 4H + 2H, $NCH_2$ + $CONHCH_2CH_2N$), 1.28 (t, $J$ = 7.0 Hz, 3H, $OCH_2CH_3$). Anal. ($C_{32}H_{34}ClN_5O_4$) C, H, N.

#### 6.1.41. 2-[[[[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-amino]carbonyl]amino]-5-[4-(phenylmethoxy)phenyl]-1*H*-pyrrole-3-carboxylic acid ethyl ester (15e)

Starting from derivative **14c** and 1-(2-methoxyphenyl)piperazine, compound **15e** was obtained as crude material. The mixture was purified by column chromatography (Silica Gel 60, 230–400 mesh, Merck) using EtOAc (100%) as eluant to afford the pure title compound (71%): mp 156–158 °C; IR (KBr, selected lines) $cm^{-1}$ 3000, 2935, 2813, 2362, 1643, 1546, 1499, 1375, 1242, 1093, 748; $^1H$ NMR (DMSO-$d_6$) $\delta$ 11.26 (br s, 1H, NH which exchanges with $D_2O$), 9.23 (br s, 1H, N*H*CONHCH$_2$ which exchanges with $D_2O$), 7.70 (br t, 1H, NHCON*H*CH$_2$ which exchanges with $D_2O$), 7.54–7.29 (m, 7H, aromatic), 7.08–6.83 (m, 6H, aromatic), 6.50 (s, 1H, pyrrole), 5.11 (s, 2H, $CH_2O$), 4.21 (q, $J$ = 7.0 Hz, 2H, O*CH*$_2$CH$_3$), 3.77 (s, 3H, $CH_3$), 3.33–3.24 (m, 2H, NHC*H*$_2$), 3.05–2.95 (m, 4H, $NCH_2$), 2.58–2.48 (m, 4H + 2H, $NCH_2$ + $CONHCH_2CH_2N$), 1.28 (t, $J$ = 7.0 Hz, 3H, $OCH_2CH_3$). Anal. ($C_{34}H_{39}N_5O_5$) C, H, N.

#### 6.1.42. 2-[[[[2-[4-(2-Chlorophenyl)-1-piperazinyl]ethyl]-amino]carbonyl]amino]-5-[4-(phenylmethoxy)phenyl]-1*H*-pyrrole-3-carboxylic acid ethyl ester (15f)

Starting from derivative **14c** and 1-(2-chlorophenyl)piperazine hydrochloride, compound **15f** was obtained as crude material. The mixture was purified by column chromatography (Silica Gel 60, 230–400 mesh, Merck) using EtOAc (100%) as eluant to afford the pure title compound (73%): mp 177–179 °C; IR (KBr, selected lines) $cm^{-1}$ 3006, 2861, 1643, 1543, 1347, 1261, 1088, 722; $^1H$ NMR (DMSO-$d_6$) $\delta$ 11.27 (br s, 1H, NH which exchanges with $D_2O$), 9.23 (br s, 1H, N*H*CONHCH$_2$ which exchanges with $D_2O$),

7.70 (br t, 1H, NHCON*H*$CH_2$ which exchanges with $D_2O$), 7.52–6.94 (m, 13H, aromatic), 6.51 (d, $^4J$ = 2.4 Hz, 1H, pyrrole), 5.11 (s, 2H, $CH_2O$), 4.22 (q, $J$ = 7.0 Hz, 2H, O*C*$H_2CH_3$), 3.34–3.25 (m, 2H, NH*C*$H_2$), 3.10–2.97 (m, 4H, $NCH_2$), 2.65–2.48 (m, 4H + 2H, $NCH_2$ + CONHC$H_2$*C*$H_2$N), 1.28 (t, $J$ = 7.0 Hz, 3H, OC$H_2$*C*$H_3$). Anal. ($C_{33}H_{36}ClN_5O_4$) C, H, N.

### 6.1.43. 2-[[[[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]-amino]carbonyl]amino]-5-[4-(2-phenoxyethoxy)phenyl]-1*H*-pyrrole-3-carboxylic acid ethyl ester (15g)

Starting from derivative **14d** and 1-(2-methoxyphenyl)piperazine, compound **15g** was obtained as crude material. The mixture was purified by column chromatography (Silica Gel 60, 230–400 mesh, Merck) using EtOAc (100%) as eluant to afford the pure title compound (65%): mp 240–242 °C; IR (KBr, selected lines) $cm^{-1}$ 2935, 2810, 1639, 1598, 1497, 1451, 1239, 1090, 753, 691; $^1H$ NMR (DMSO-$d_6$) $\delta$ 11.27 (br s, 1H, NH which exchanges with $D_2O$), 9.23 (br s, 1H, N*H*CONHC$H_2$ which exchanges with $D_2O$), 7.70 (br t, 1H, NHCON*H*C$H_2$ which exchanges with $D_2O$), 7.55–7.24 (m, 4H, aromatic), 7.09–6.82 (m, 9H, aromatic), 6.51 (s, 1H, pyrrole), 4.31 (s, 2H + 2H, OC$H_2$C$H_2$O), 4.26 (q, $J$ = 7.0 Hz, 2H, O*C*$H_2CH_3$), 3.80 (s, 3H, $CH_3$), 3.39–3.22 (m, 2H, NH*C*$H_2$), 3.11–2.92 (m, 4H, $NCH_2$), 2.62–2.48 (m, 4H + 2H, $NCH_2$ + CONHC$H_2$*C*$H_2$N), 1.31 (t, $J$ = 7.0 Hz, 3H, OC$H_2$*C*$H_3$). Anal. ($C_{35}H_{41}N_5O_6$) C, H, N.

### 6.1.44. 2-[[[[2-[4-(2-Chlorophenyl)-1-piperazinyl]ethyl]-amino]carbonyl]amino]-5-[4-(2-phenoxyethoxy)phenyl]-1*H*-pyrrole-3-carboxylic acid ethyl ester (15h)

Starting from derivative **14d** and 1-(2-chlorophenyl)piperazine hydrochloride, compound **15h** was obtained as crude material. The mixture was purified by column chromatography (Silica Gel, 60 230–400 mesh, Merck) using EtOAc (100%) as eluant to afford the pure title compound (63%): mp 245–247 °C; IR (KBr, selected lines) $cm^{-1}$ 2978, 2957, 1640, 1599, 1484, 1449, 1240, 1081, 757, 691; $^1H$ NMR (DMSO-$d_6$) $\delta$ 11.26 (br s, 1H, NH which exchanges with $D_2O$), 9.23 (br s, 1H, N*H*CONHC$H_2$ which exchanges with $D_2O$), 7.70 (br t, 1H, NHCON*H*C$H_2$ which exchanges with $D_2O$), 7.54–6.89 (m, 13H, aromatic), 6.51 (s, 1H, pyrrole), 4.31 (s, 2H + 2H, OC$H_2$C$H_2$O), 4.22 (q, $J$ = 7.0 Hz, 2H, O*C*$H_2CH_3$), 3.36–3.26 (m, 2H, NH*C*$H_2$), 3.08–2.89 (m, 4H, $NCH_2$), 2.64–2.51 (m, 4H + 2H, $NCH_2$ + CONHC$H_2$*C*$H_2$N), 1.28 (t, $J$ = 7.0 Hz, 3H, OC$H_2$*C*$H_3$). Anal. ($C_{34}H_{38}ClN_5O_5$) C, H, N.

### 6.1.45. 2-[[[[2-[4-(2-Chlorophenyl)-1-piperazinyl]ethyl]-amino]carbonyl]amino]-5-phenyl-1-(phenylmethyl)-1*H*-pyrrole-3-carboxylic acid ethyl ester (15i)

Starting from derivative **14f** and 1-(2-chlorophenyl)piperazine hydrochloride, compound **15i** was obtained as crude material. The mixture was purified by column chromatography (Silica Gel 60, 230–400 mesh, Merck) using EtOAc (100%) as eluant to afford the pure title compound (79%): mp 162–164 °C; IR (KBr, selected lines) $cm^{-1}$ 3858, 3377, 2817, 1692, 1631, 1519, 1480, 1281, 1236, 933, 787; $^1H$ NMR (DMSO-$d_6$) $\delta$ 11.91 (br s, 1H, NH which exchanges with $D_2O$), 7.70–7.59 (m, 2H, aromatic), 7.40–6.80 (m, 12H + 1H, aromatic + pyrrole), 5.80 (br t, 1H, NCONH), 4.79 (s, 2H, $CH_2Ar$), 4.07 (q, $J$ = 7.0 Hz, 2H, O*C*$H_2CH_3$), 3.22–3.10 (m, 2H, NH*C*$H_2$), 2.85–2.69 (m, 4H, $NCH_2$), 2.50–2.35 (m, 4H + 2H, $NCH_2$ + CONHC$H_2$*C*$H_2$N), 1.20 (t, $J$ = 7.0 Hz, 3H, OC$H_2$*C*$H_3$). Anal. ($C_{33}H_{36}ClN_5O_3$) C, H, N.

### 6.1.46. 5-[4-(1,1'-Biphenyl)]-2-[[[(2-chloroethyl)amino]-carbonyl]amino]-1*H*-pyrrole-3-carboxylic acid ethyl ester (14a)

A suspension of 2-amino-5-[4-(1,1'-biphenyl)]-1*H*-pyrrole-3-carboxylic acid ethyl ester (**13a**) (3.10 mmol, 0.950 g) and 2-chloroethyl isocyanate (4.65 mmol, 0.397 mL) in 7 mL of toluene was stirred for 6 h at reflux. The reaction mixture was then evaporated in vacuo. The obtained crude material was purified by column chromatography (Silica Gel 60, 230–400 mesh, Merck) using cyclohexane/EtOAc (7:3) as eluant, to afford the title compound (**14a**) as pure product (0.781 g, 61%): mp 124–126 °C; IR (KBr, selected lines) $cm^{-1}$ 3140, 1600, 1443, 1348, 1264, 1112, 760, 607; $^1H$ NMR (DMSO-$d_6$) $\delta$ 11.36 (br s, 1H, NH which exchanges with $D_2O$), 9.31 (br s, 1H, N*H*CONHC$H_2$ which exchanges with $D_2O$), 8.02 (t, $J$ = 5.8 Hz, 1H, NHCON*H*C$H_2$ which exchanges with $D_2O$), 7.74–7.63 (m, 6H, aromatic), 7.55–7.31 (m, 3H, aromatic), 6.73 (d, $^4J$ = 3.0 Hz, 1H, pyrrole), 4.25 (q, $J$ = 7.0 Hz, 2H, O*C*$H_2CH_3$), 3.72 (t, $J$ = 6.0 Hz, 2H, $CH_2Cl$), 3.54–3.43 (m, 2H, NH*C*$H_2$), 1.30 (t, $J$ = 7.0 Hz, 3H, OC$H_2$*C*$H_3$). Anal. ($C_{22}H_{22}ClN_3O_3$) C, H, N.

Using this procedure the subsequent compounds were obtained.

### 6.1.47. 2-[[[(2-Chloroethyl)amino]carbonyl]amino]-5-(4-phenoxyphenyl)-1*H*-pyrrole-3-carboxylic acid ethyl ester (14b)

Starting from derivative **13b**, the title compound (**14b**) was obtained as a pure product (0.985 g, 74%): mp 99–101 °C; IR (KBr, selected lines) $cm^{-1}$ 3189, 3270, 1643, 1580, 1485, 1370, 1244, 1099, 839, 693; $^1H$ NMR (DMSO-$d_6$) $\delta$ 11.28 (br s, 1H, NH which exchanges with $D_2O$), 9.26 (br s, 1H, N*H*CONHC$H_2$ which exchanges with $D_2O$), 7.99 (t, $J$ = 5.8 Hz, 1H, NHCON*H*C$H_2$ which exchanges with $D_2O$), 7.69–7.59 (m, 2H, aromatic), 7.49–7.32 (m, 2H, aromatic), 7.21–6.91 (m, 5H, aromatic), 6.60 (d, $^4J$ = 3.0 Hz, 1H, pyrrole), 4.23 (q, $J$ = 7.0 Hz, 2H, O*C*$H_2CH_3$), 3.70 (t, $J$ = 6.0 Hz, 2H, $CH_2Cl$), 3.58–3.34 (m, 2H, NH*C*$H_2$), 1.29 (t, $J$ = 7.0 Hz, 3H, OC$H_2$*C*$H_3$). Anal. ($C_{22}H_{22}ClN_3O_4$) C, H, N.

### 6.1.48. 2-[[[(2-Chloroethyl)amino]carbonyl]amino]-5-[4-(phenylmethoxy)phenyl]-1*H*-pyrrole-3-carboxylic acid ethyl ester (14c)

Starting from derivative **13c**, the title compound (**14c**) was obtained as a pure product (1.180 g, 86%): mp 152–154 °C; IR (KBr, selected lines) $cm^{-1}$ 3027, 2362, 1639, 1558, 1265, 1096, 776; $^1H$ NMR (DMSO-$d_6$) $\delta$ 11.21 (br s, 1H, NH which exchanges with $D_2O$), 9.25 (br s, 1H, N*H*CONHC$H_2$ which exchanges with $D_2O$), 7.97 (t, $J$ = 5.8 Hz, 1H, NHCON*H*C$H_2$ which exchanges with $D_2O$), 7.69–7.59 (m, 2H, aromatic), 7.53–7.29 (m, 2H, aromatic), 7.05–6.95 (m, 5H, aromatic), 6.52 (d, $^4J$ = 2.8 Hz, 1H, pyrrole), 5.11 (s, 2H, $CH_2O$), 4.22 (q, $J$ = 7.0 Hz, 2H, O*C*$H_2CH_3$), 3.70 (t, $J$ = 6.0 Hz, 2H, $CH_2Cl$), 3.54–3.43 (m, 2H, NH*C*$H_2$), 1.28 (t, $J$ = 7.0 Hz, 3H, OC$H_2$*C*$H_3$). Anal. ($C_{23}H_{24}ClN_3O_4$) C, H, N.

### 6.1.49. 2-[[[(2-Chloroethyl)amino]carbonyl]amino]-5-[4-(2-phenoxyethoxy)phenyl]-1*H*-pyrrole-3-carboxylic acid ethyl ester (14d)

Starting from derivative **13d**, the title compound (**14d**) was obtained as a pure product (0.873 g, 60%): mp 140–142 °C; IR (KBr, selected lines) $cm^{-1}$ 2935, 1632, 1556, 1495, 1370, 1235, 1172, 1065, 940, 777; $^1H$ NMR (DMSO-$d_6$) $\delta$ 11.20 (br s, 1H, NH which exchanges with $D_2O$), 9.24 (br s, 1H, N*H*CONHC$H_2$ which exchanges with $D_2O$), 7.97 (t, $J$ = 5.8 Hz, 1H, NHCON*H*C$H_2$ which exchanges with $D_2O$), 7.55–7.21 (m, 4H, aromatic), 7.03–6.88 (m, 5H, aromatic), 6.52 (d, $^4J$ = 3.0 Hz, 1H, pyrrole), 4.31 (s, 2H + 2H, OC$H_2$C$H_2$O), 4.23 (q, $J$ = 7.0 Hz, 2H, O*C*$H_2CH_3$), 3.70 (t, $J$ = 6.0 Hz, 2H, $CH_2Cl$), 3.54–3.42 (m, 2H, NH*C*$H_2$), 1.29 (t, $J$ = 7.0 Hz, 3H, OC$H_2$*C*$H_3$). Anal. ($C_{24}H_{26}ClN_3O_5$) C, H, N.

### 6.1.50. 2-[[[(2-Chloroethyl)amino]carbonyl]amino]-4,5-diphenyl-1*H*-pyrrole-3-carboxylic acid ethyl ester (14e)

Starting from derivative **13e**,[28] the title compound was obtained as a pure product (0.794 g, 62%): mp 134–136 °C; IR (KBr, selected lines) $cm^{-1}$ 2984, 1611, 1445, 1290, 1230, 1138, 1096, 762, 699; $^1H$ NMR (DMSO-$d_6$) $\delta$ 11.28 (br s, 1H, NH which exchanges with $D_2O$), 9.42 (br s, 1H, N*H*CONHC$H_2$ which exchanges

with $D_2O$), 8.09 (t, $J$ = 5.8 Hz, 1H, NHCON*H*$CH_2$ which exchanges with $D_2O$), 7.33–7.01 (m, 10H, aromatic), 3.95 (q, $J$ = 7.0 Hz, 2H, O*C*$H_2$CH$_3$), 3.71 (t, $J$ = 6.0 Hz, 2H, $CH_2$Cl), 3.56–3.42 (m, 2H, NH*C*$H_2$), 0.88 (t, $J$ = 7.0 Hz, 3H, OCH$_2$*C*$H_3$). Anal. ($C_{22}H_{22}ClN_3O_3$) C, H, N.

#### 6.1.51. 2-[[[(2-Chloroethyl)amino]carbonyl]amino]-5-phenyl-1-(phenylmethyl)-1*H*-pyrrole-3-carboxylic acid ethyl ester (14f)

Starting from derivative **13f**, the title compound (**14f**) was obtained as a pure product (0.869 g, 66%): mp 123–125 °C; IR (KBr, selected lines) cm$^{-1}$ 2878, 1679, 1502, 1250, 1221, 1026, 757, 700; $^1$H NMR (DMSO-$d_6$) $\delta$ 11.86 (br s, 1H, NH which exchanges with $D_2O$), 7.68–7.60 (m, 2H, aromatic), 7.41–7.13 (m, 8H, aromatic), 6.78 (d, $^4J$ = 2.8 Hz, 1H, pyrrole), 6.30 (t, $J$ = 5.8 Hz, 1H, NHCON*H*$CH_2$ which exchanges with $D_2O$), 4.77 (s, 2H, $CH_2$Ar), 4.05 (q, $J$ = 7.0 Hz, 2H, O*C*$H_2$CH$_3$), 3.54 (t, $J$ = 6.0 Hz, 2H, $CH_2$Cl), 3.37–3.25 (m, 2H, NH*C*$H_2$), 1.19 (t, $J$ = 7.0 Hz, 3H, OCH$_2$*C*$H_3$). Anal. ($C_{23}H_{24}ClN_3O_3$) C, H, N.

#### 6.1.52. 2-[[[(2-Chloroethyl)amino]carbonyl]amino]-5-(4-methoxyphenyl)-1-[(2-methoxyphenyl)methyl]-1*H*-pyrrole-3-carboxylic acid ethyl ester (14g)

Starting from derivative **13g**, the title compound (**14g**) was obtained as a pure product (1.07 g, 71%): mp 161–163 °C; IR (KBr, selected lines) cm$^{-1}$ 3156, 1694, 1640, 1466, 1245, 1142, 1026, 760; $^1$H NMR (DMSO-$d_6$) $\delta$ 11.68 (br s, 1H, NH which exchanges with $D_2O$), 7.63–7.39 (m, 3H, aromatic), 7.21–7.11 (m, 1H, aromatic), 6.97–6.78 (m, 4H, aromatic), 6.65 (d, $^4J$ = 2.8 Hz, 1H, pyrrole), 6.24 (t, $J$ = 5.8 Hz, 1H, NHCON*H*$CH_2$ which exchanges with $D_2O$), 4.73 (s, 2H, $CH_2$Ar), 4.08 (q, $J$ = 7.0 Hz, 2H, O*C*$H_2$CH$_3$), 3.76 (s, 3H, $CH_3$), 3.60 (s, 3H, $CH_3$), 3.53 (t, $J$ = 6.0 Hz, 2H, $CH_2$Cl), 3.37–3.29 (m, 2H, NH*C*$H_2$), 1.20 (t, $J$ = 7.0 Hz, 3H, OCH$_2$*C*$H_3$). Anal. ($C_{25}H_{28}ClN_3O_5$) C, H, N.

#### 6.1.53. 2-Amino-5-[4-(1,1'-biphenyl)]-1*H*-pyrrole-3-carboxylic acid ethyl ester (13a)[29]

Ammonium chloride (**11a**) (0.820 g, 15.3 mmol) was added to a suspension of 3-ethoxy-3-iminopropanoic acid ethyl ester hydrochloride (2.00 g, 10.2 mmol) and $K_2CO_3$ (2.47 g, 17.9 mmol) in 10 mL of anhydrous ethanol and the reaction mixture was stirred at 22 °C for 24 h. The obtained suspension was used in the subsequent step without isolating 3-amino-3-iminopropanoic acid ethyl ester from the reaction environment. 1-(1,1'-Biphenyl-4-yl)-2-bromoethanone (**12a**) (2.18 g, 10.2 mmol) and $K_2CO_3$ (0.706 g, 5.11 mmol) was added to the previously obtained suspension and the reaction mixture was refluxed for 2 h. After this period, the solvent was evaporated at reduced pressure, the residue was diluted with NaOH 1 N (100 mL) and extracted with EtOAc (3 × 100 mL). The combined organic layers were washed with brine, dried over anhydrous $Na_2SO_4$, filtered, and evaporated in vacuo. The obtained crude material was purified by column chromatography (Silica Gel 60, 230–400 mesh, Merck) using cyclohexane/EtOAc (6:4) as eluant, to afford the title compound (**13a**) as pure product (1.36 g, 43%): mp 179–181 °C; IR (KBr, selected lines) cm$^{-1}$ 3052, 1644, 1549, 1377, 1241, 1190, 1077, 838, 762; $^1$H NMR (DMSO-$d_6$) $\delta$ 10.86 (br s, 1H, NH which exchanges with $D_2O$), 7.72–7.30 (m, 9H, aromatic), 6.55 (d, $^4J$ = 2.6 Hz, 1H, pyrrole), 5.73 (br s, 2H, $NH_2$ which exchanges with $D_2O$), 4.15 (q, $J$ = 7.0 Hz, 2H, O*C*$H_2$CH$_3$), 1.26 (t, $J$ = 7.0 Hz, 3H, OCH$_2$*C*$H_3$). Anal. ($C_{19}H_{18}N_2O_2$) C, H, N.

Using this procedure the subsequent compounds were obtained.

#### 6.1.54. 2-Amino-5-(4-phenoxyphenyl)-1*H*-pyrrole-3-carboxylic acid ethyl ester (13b)

Starting from derivatives **11a** and **12b**,[31] the title compound (**13b**) was obtained as a pure product (1.25 g, 38%): mp 98–100 °C; IR (KBr, selected lines) cm$^{-1}$ 2914, 1665, 1570, 1501, 1378, 1240 1141, 999, 776; $^1$H NMR (DMSO-$d_6$) $\delta$ 10.72 (br s, 1H, NH which exchanges with $D_2O$), 7.55–7.32 (m, 4H, aromatic), 7.18–6.91 (m, 5H, aromatic), 6.41 (d, $^4J$ = 3.0 Hz, 1H, pyrrole), 5.66 (br s, 2H, $NH_2$ which exchanges with $D_2O$), 4.14 (q, $J$ = 7.0 Hz, 2H, O*C*$H_2$CH$_3$), 1.25 (t, $J$ = 7.0 Hz, 3H, OCH$_2$*C*$H_3$). Anal. ($C_{19}H_{18}N_2O_3$) C, H, N.

#### 6.1.55. 2-Amino-5-[4-(phenylmethoxy)phenyl]-1*H*-pyrrole-3-carboxylic acid ethyl ester (13c)[30]

Starting from derivatives **11a** and **12c**,[32] the title compound (**13c**) was obtained as a pure product (1.19 g, 35%): mp 175–177 °C; IR (KBr, selected lines) cm$^{-1}$ 2910, 1661, 1567, 1499, 1380, 1279, 1182, 1133, 1005, 775; $^1$H NMR (DMSO-$d_6$) $\delta$ 10.66 (br s, 1H, NH which exchanges with $D_2O$), 7.46–7.30 (m, 7H, aromatic), 7.01–6.91 (m, 2H, aromatic), 6.31 (d, $^4J$ = 2.6 Hz, 1H, pyrrole), 5.62 (br s, 2H, $NH_2$ which exchanges with $D_2O$), 5.09 (s, 2H, $CH_2O$), 4.13 (q, $J$ = 7.0 Hz, 2H, O*C*$H_2$CH$_3$), 1.24 (t, $J$ = 7.0 Hz, 3H, OCH$_2$*C*$H_3$). Anal. ($C_{20}H_{20}N_2O_3$) C, H, N.

#### 6.1.56. 2-Amino-5-[4-(2-phenoxyethoxy)phenyl]-1*H*-pyrrole-3-carboxylic acid ethyl ester (13d)

Starting from derivatives **11a** and **12d**, the title compound (**13d**) was obtained as a pure product (1.34 g, 36%): mp 144–146 °C; IR (KBr, selected lines) cm$^{-1}$ 2879, 1650, 1569, 1498, 1243, 1185, 1075, 939, 833, 755; $^1$H NMR (DMSO-$d_6$) $\delta$ 10.64 (br s, 1H, NH which exchanges with $D_2O$), 7.47–7.24 (m, 4H, aromatic), 7.02–6.89 (m, 5H, aromatic), 6.31 (d, $^4J$ = 2.6 Hz, 1H, pyrrole), 5.61 (br s, 2H, $NH_2$ which exchanges with $D_2O$), 4.30 (s, 2H + 2H, OCH$_2$-CH$_2$O), 4.13 (q, $J$ = 7.0 Hz, 2H, O*C*$H_2$CH$_3$), 1.24 (t, $J$ = 7.0 Hz, 3H, OCH$_2$*C*$H_3$). Anal. ($C_{21}H_{22}N_2O_4$) C, H, N.

#### 6.1.57. 2-Amino-5-phenyl-1-(phenylmethyl)-1*H*-pyrrole-3-carboxylic acid ethyl ester (13f)

Starting from derivatives **11b** and **12f**, the title compound (**13f**) was synthesized by using phenylmethanamine as starting commercial product instead of ammonium chloride. The title derivative was obtained as a pure product (1.58 g, 48%): mp 125–127 °C; IR (KBr, selected lines) cm$^{-1}$ 3061, 1649, 1448, 1237, 1184, 1112, 756, 695. $^1$H NMR (DMSO-$d_6$) $\delta$ 10.59 (br s, 1H, NH which exchanges with $D_2O$), 7.75–7.50 (m, 2H, aromatic), 7.48–7.14 (m, 8H, aromatic), 6.76 (t, $J$ = 6.6 Hz, 1H, NH which exchanges with $D_2O$), 6.52 (s, 1H, pyrrole), 4.62 (d, $J$ = 6.6 Hz, 2H, NH*C*$H_2$), 4.15 (q, $J$ = 7.0 Hz, 2H, O*C*$H_2$CH$_3$), 1.16 (t, $J$ = 7.0 Hz, 3H, OCH$_2$*C*$H_3$). Anal. ($C_{20}H_{20}N_2O_2$) C, H, N.

#### 6.1.58. 2-Amino-5-(4-methoxyphenyl)-1-[(2-methoxyphenyl)-methyl]-1*H*-pyrrole-3-carboxylic acid ethyl ester (13g)

Starting from derivatives **11c** and **12g**, the title compound (**13g**) was synthesized by using (2-methoxyphenyl)methanamine as starting commercial product instead of ammonium chloride. The title derivative was obtained as a pure product (1.57 g, 40%): mp 130–131 °C; IR (KBr, selected lines) cm$^{-1}$ 2982, 2899, 2830, 1649, 1606,1499, 1459, 1419, 1237, 1104, 1026, 829, 774; $^1$H NMR (DMSO-$d_6$) $\delta$ 10.50 (br s, 1H, NH which exchanges with $D_2O$), 7.55–7.48 (m, 2H, aromatic), 7.33–7.19 (m, 2H, aromatic), 7.06–6.85 (m, 4H, aromatic), 6.67 (t, $J$ = 6.8 Hz, 1H, NH which exchanges with $D_2O$), 6.34 (d, $^4J$ = 2.2 Hz, 1H, pyrrole), 4.54 (d, $J$ = 6.8 Hz, 2H, NH*C*$H_2$), 4.13 (q, $J$ = 7.0 Hz, 2H, O*C*$H_2$CH$_3$), 3.87 (s, 3H, $CH_3$), 3.84 (s, 3H, $CH_3$), 1.19 (t, $J$ = 7.0 Hz, 3H, OCH$_2$*C*$H_3$). Anal. ($C_{22}H_{24}N_2O_4$) C, H, N.

#### 6.1.59. 2-Bromo-1-[4-(2-phenoxyethoxy)phenyl]-ethanone (12d)

A mixture of (2-chloroethoxy)benzene (3.00 g, 24.0 mmol), 1-(4-hydroxyphenyl)-ethanone (3.76 g, 24.0 mmol), $K_2CO_3$ (6.09 g,

48.08 mmol), and a catalytic amount of KI in 50 mL of dry acetone was refluxed for 48 h. After this period, the solvent was evaporated at reduced pressure, the residue was diluted with EtOAc (200 mL) and washed subsequently with NaOH 1 N (3 × 100 mL), HCl 1 N (1 × 100 mL) and brine. The organic layer was dried over anhydrous $Na_2SO_4$, filtered, and evaporated in vacuo. The obtained crude 1-[4-(2-phenoxyethoxy)phenyl]ethanone (15.17 mmol) was dissolved in 10 mL of glacial acetic acid, bromine (15.17 mmol) was added dropwise and the reaction mixture was stirred at 22 °C for 1 h. The obtained reaction mixture was dropped in saturated $NaHCO_3$, extracted with EtOAc (3 × 30 mL). The combined organic layers were dried over $Na_2SO_4$, filtered and evaporated. The obtained crude 2-bromo-1-[4-(2-phenoxyethoxy)phenyl]-ethanone (**12d**) was used in the subsequent step without any further purification. A small sample of the crude **12d** was purified by column chromatography (Silica Gel, 60 230–400 mesh, Merck) using cyclohexane/EtOAc (9:1) as eluant, to afford the title compound (**12d**) as pure product: mp 90–92 °C; IR (KBr, selected lines) $cm^{-1}$ 2932, 1689, 1598,1491, 1304, 1264, 1243, 1174, 1063, 941, 828, 760; $^1H$ NMR (DMSO-$d_6$) $\delta$ 8.05–7.95 (m, 2H, aromatic), 7.37–7.24 (m, 2H, aromatic), 7.20–7.07 (m, 2H, aromatic), 7.01–6.92 (m, 3H, aromatic), 4.86 (s, 2H, $CH_2Br$), 4.48–4.30 (m, 2H + 2H, $OCH_2CH_2O$). Anal. ($C_{16}H_{15}O_3$) C, H, N.

### 6.2. Computational methods

All compounds considered in the present study were built by ISIS/Draw[38] and automatically converted to 3D by AMMP[39,40] software included in VEGA ZZ[41] package, using the SP4[42] force field. The compounds were considered in their protonated forms since they may be involved in ligand recognition. Finally, the structures were optimized by semi-empirical calculations (Mopac 2009,[43] PM6 PRECISE MMOK keywords) and included in a SQLite[44] database which was exploited by GriDock,[45] a parallel software based on AutoDock 4.0[46] which we have developed for efficient and easy virtual screening analyses of large molecular databases exploiting multi-core architectures.

The three dimensional structures of the human $\alpha_{1a}$-AR[33] and the human 5-$HT_{1A}$ serotoninergic receptor[34] were taken by previous studies in which they were generated by homology techniques and validated by docking calculations. In detail, for the $\alpha_{1a}$-AR, the grid box was set to include all residues within a 12 Å radius sphere around the Asp-106, which plays a pivotal role in the interaction with cathecolamines,[35] thus comprising the entire binding cavity. The resolution of the grid was 60 × 60 × 60 points with a grid spacing of 0.450 Å. For the 5-$HT_{1A}$ serotoninergic receptor, the best model was selected from a set of 100 structures according to the method explained by Nowak et al.[34] The grid box was generated including all residues within a 12 Å radius sphere around the Asp-116, keeping the same parameters as for the $\alpha_{1a}$-AR.

Each substrate was docked into the grids with the Lamarckian algorithm as implemented in AutoDock. For the docking simulations, the flexible bonds of the ligand were automatically recognized by *GriDock* and left free to rotate. The genetic-based algorithm ran 20 simulations per substrate with 2000,000 energy evaluations and a maximum number of generations of 27,000. The crossover rate was increased to 0.8, and the number of individuals in each population to 150. All other parameters were left at the AutoDock default settings.

The docking results were ranked considering both AutoDock scores and the closeness between Asp-106 ($\alpha_{1a}$-AR) or Asp-116 (5-$HT_{1A}$ receptor) and the protonated function. The best complexes were minimized by NAMD 2.7,[47] keeping fixed all atoms outside a 12 Å radius sphere around the bound substrate to favor the mutual adaptability between ligand and receptor. The optimized complexes were then used to re-calculate AutoDock docking scores, FRED[48] scores, VEGA energy scores (CHARMM 22,[49] CHARMM 36[50] and CVFF[51]) and $MLP_{InS}$[36] that we have recently developed to account for hydrophobic interactions, the importance of which is well documented in ligand recognition.[52] In the statistical analyses, a small set of representative physicochemical properties of the bound ligands (as computed by VEGA, e.g., virtual log *P*, PSA, SAS, volume, molecular weight and dipole moment) were also taken into account.

To better visualize the hydrophilic/lipophilic complementarity between ligand and receptor, the MLP the interaction score as calculated for each ligand atom was projected on the solid Van der Waals surface and each surface vertex was coloured using a colour ramp in order to highlight the regions with high (red regions) or low (blue regions) complementarity with the receptor pocket.

### 6.3. Evaluation of compounds 5a–d, 10a–f, and 16a–n in binding assays

Male CRL:CD(SD)BR-COBS rats weighing about 150 g were killed by decapitation and their brains were rapidly dissected (hippocampus for 5-$HT_{1A}$; cortex for $\alpha_1$-ARs), frozen, and stored at −80 °C until the day of assay. Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national (D.L. n. 116, G.U., suppl. 40, Feb. 18, 1992) and international laws and policies (EEC Council Directive 86/609, OJ L 358, 1, Dec. 12, 1987; Guide for the Care and Use of Laboratory Animals, US National Research Council, 1996).

Tissue was homogenized in about 50 volumes of ice-cold 50 mM Tris–HCl buffer (pH 7.4) using an Ultra Turrax TP-1810 (2 × 20 s) and centrifuged at 50,000*g* for 10 min (Beckman model J-21B refrigerated centrifuge). The pellet was resuspended in the same volume of fresh buffer, incubated at 37 °C for 10 min, and centrifuged again at 50,000*g* for 10 min. The pellet was then washed once by resuspension in fresh buffer and centrifuged as before. The pellet was then resuspended in the appropriate incubation buffer (50 mM Tris–HCl, pH 7.7 containing 10 μM pargyline and 4 mM $CaCl_2$ for 5-$HT_{1A}$ receptors; 50 mM Tris–HCl , pH 7.7 containing 10 μM pargyline and 0.1% ascorbic acid for $\alpha_1$-ARs) just before the binding assay.

Binding assays were done as previously described.[53] Briefly, the following incubation conditions were used. 5-$HT_{1A}$: [$^3$H]-8-OH-DPAT (sp act. 157 Ci/mmol, NEN) final concentration 1 nM, 30 min, 25 °C (nonspecific binding: 5-HT 10 μM). $\alpha_1$-ARs: [$^3$H]prazosin (sp act. 71.8 Ci/mmol, NEN) final concentration 0.2 nM, 30 min, 25 °C (nonspecific binding: phentolamine 3 μM).

Incubations were stopped by rapid filtration under vacuum through GF/B filters which were then washed with 12 mL (4 × 3 times) of ice-cold 50 mM Tris–HCl (pH 7.4) or 50 mM Hepes-HCl (pH 7.5) using a Brandel M-48R apparatus and counted in 4 mL of Filter Count (Packard) in a LKB 1214 RACKBETA liquid scintillation spectrometer with counting efficiency about 60%. Dose inhibition curves were analyzed by the 'ALLFIT' program to obtain the concentration of unlabeled drugs that inhibited ligand binding by 50%.[54] The $K_i$ values were derived from the $IC_{50}$ values.[55]

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### Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.06.043.

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