European Journal of Medicinal Chemistry 46 (2011) 1207–1221

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and pharmacological evaluation of bivalent antagonists of the nociceptin opioid receptor

Maria Rosaria Del Giudice*, Anna Borioni, Giuditta Bastanzio, Maria Sbraccia, Carlo Mustazza, Isabella Sestili

Dipartimento del Farmaco, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Roma, Italy

ARTICLE INFO

Article history:
Received 11 October 2010
Received in revised form
17 January 2011
Accepted 25 January 2011
Available online 3 February 2011

Keywords:
Synthesis
Quinoline
Bivalent ligand
NOP receptor antagonism

ABSTRACT

Bivalent ligands constituted by two identical pharmacophores structurally related to the Nociceptin Opioid Receptor (NOPr) antagonist JTC-801 were synthesized and their binding affinities for NOPr were evaluated. The novel ligands are formed by two modified JTC-801 units linked by di-iminic and di-aminic spacers with length ranging from three to ten methylene units. Moreover, the synthesis and the pharmacological characterization were extended to the corresponding univalent ligands. The latter compounds consisted in a single modified JTC-801 unit and an alkyl or alkylamino or alkylimino tail. The purpose of this study is to feature the location and surroundings of the allosteric binding site(s) of pharmacophores containing the 4-aminoquinoline structure. Most important, the bivalent ligands were exploited to reveal the eventual occurrence of a supramolecular receptorial architecture of the NOPr.

All the bivalent derivatives **4** and **5** proved to be active in the nanomolar range with no outstanding dependence on the chain length. They showed potencies from three to ten times higher than the corresponding monomers. Consequently, results clearly indicated a positive role of the second pharmacophore in the ligand–protein interaction. The pharmacological profile of the monomers **7** and **8** clarified the contribution of the linker chain to NOP receptor affinity and suggested the presence of a lipophilic acidic site neighbouring the binding site of the JTC-like ligands.

Selectivity of saturated compounds **5**, **7**, and **8** was tested by binding experiments on δ, κ and μ opioid receptors. Results indicated a general loss of selectivity as compared to JTC-801. In the [^{35}S]GTPγS binding assay, all the compounds revealed antagonistic properties at the NOP Receptor.

In conclusion the present study set the basis for a systematic investigation on the structural modifications that can be introduced into novel ligands for NOPr and helped to feature the surrounds of the allosteric site of NOPr.

1. Introduction

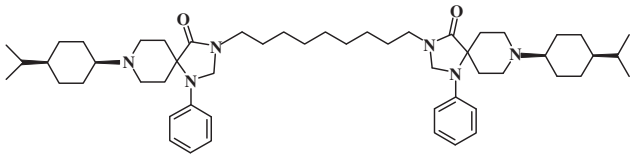
The nociceptin (NOP) receptor [1] is the most recently discovered member of the family of the opioid receptor. The NOP receptor antagonists currently attract a rapidly growing pharmacological interest. Their perspective clinical applications are as analgesics devoid of tolerance, anorexiants, memory enhancers, antidepressants and in the treatment of Parkinson's disease [2]. The first NOPr antagonist used in clinical trials was the analgesic JTC-801 [3–5], whose molecular structure is based on *N*-(4-amino-2-methylquinolin-6-yl)-2-phenoxymethylbenzamide moiety. It departs from that of the most widely studied classes of NOP ligands, namely the 4-substituted piperidines and spiropiperidines. In the interaction with the NOP receptor, JTC-801 showed peculiar characteristics associated to structural and conformational aspects [5]. In Schild analysis [5] it behaved as a non-competitive antagonist, probably bound to an allosteric site of NOPr. At present, the location and attributes of JTC-801 binding site are still undisclosed.

As not much is known about putative NOPr allosteric antagonists, we chose JTC-801 as the lead structure to generate new NOPr ligands in univalent and bivalent form.

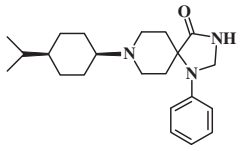
The development of bivalent ligands is nowadays a common practice in medicinal chemistry [6–9]. In particular, bifunctional ligand design and targeting of dimeric receptors were recently evidenced as big novel opportunities in the field of opioid receptor ligands. Bivalent ligands could exhibit distinct pharmacokinetic and pharmacological profiles compared to the lead univalent

* Corresponding author. Tel./fax: +39 06 49902516.
E-mail address: mariarosaria.delgiudice@iss.it (M.R. Del Giudice).

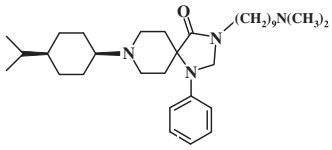
0223-5234/$ – see front matter © 2011 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2011.01.040



(Clark & Elbing LLP), IC50 =25.2 nM



Ro 646198, IC50 = 4.4 nM



(Clark & Elbing LLP), IC50 = 5.5 nM

Fig. 1. Structures of dimeric and monomeric ligands from 1,3,8-triazaspiro[4.5]decan-4-one pharmacophore.

compounds, showing unexpected effects on potency and selectivity. The two pharmacophoric moieties of bivalent ligands have been depicted to interact cooperatively with different units of the dimeric receptor [10–12].

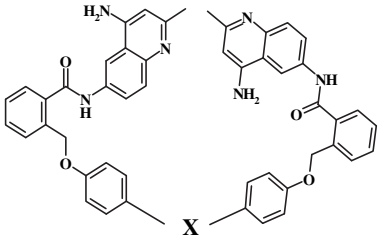
Moreover, bivalent ligands were used to achieve selectivity among central and peripheral effects of widely distributed receptors, as they often display different Blood–Brain Barrier permeation in respect to the monomeric unit. Indeed the only bivalent NOPr ligands so far reported were developed as selective agonists for the peripheral nervous system (patent by Clark & Elbing LLP) [13]. These compounds, structurally related to the orthosteric NOP ligand Ro 646198 [14], showed about 6-fold lower affinity as compared with the originator (Fig. 1).

Novel compounds **4** and **5** (Fig. 2) are the first bivalent NOPr ligands based on antagonist pharmacophores. A modification of JTC-801 structure was accomplished by inserting an iminic –CH=N–alkyl or an aminic –CH_2NH–alkyl linker between the two pharmacophoric units (Fig. 2). Aliphatic linker chains ranged from 3 to 10 methylene units with a corresponding elongation from 4.8 Å to 13.7 Å. At present, no information on the location of allosteric sites in nociceptin receptor was available in the literature. Therefore, this series of bivalent compounds covering a wide range of distances represent a starting point as probes to the existence of native dimeric forms of the NOPr.

All the novel compounds, including the univalent congeners **6**, **7** and **8** (Fig. 2), were also synthesized and tested to elucidate the features and surroundings of the binding site(s) location of 4-aminoquinoline derived pharmacophores. A structure/activity relationship of bivalent azomethines **4** and univalent azomethines **6** made possible to investigate the influence of configurational rigidity on the pharmacological properties.

Unsaturated and saturated univalent alkyl derivatives **6**, **7** were a helpful tool to factor out the contribution of the aliphatic chain to the ligand–protein interaction. Pharmacological profile of the univalent aminic compounds **8** aided in elucidating the

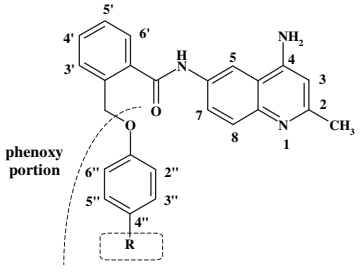
DIMERIC COMPOUNDS



4 (a-h): X = -CH=N-$(CH_2)_n$-N=CH-

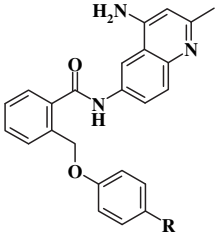
5 (a-h): X= -CH_2-NH-$(CH_2)_n$-NH-CH_2-

REFERENCE COMPOUNDS



n = 3-10

MONOMERIC COMPOUNDS



JTC-801 R = -CH_2CH_3 IC_{50} = 30 nM

3 R = -CHO IC_{50} = 207 nM

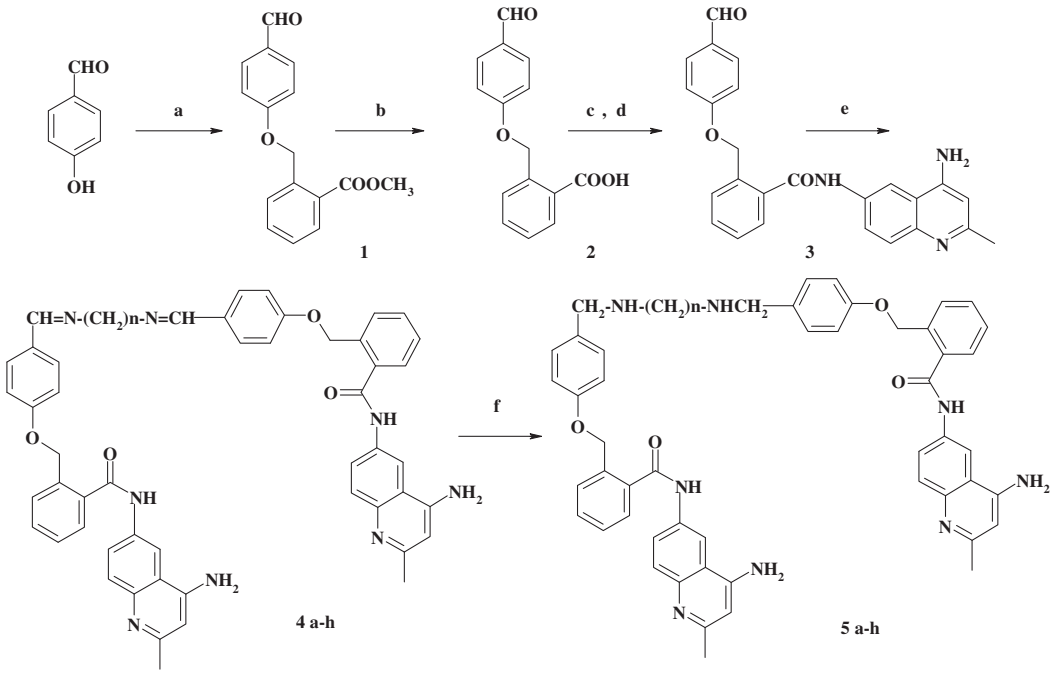
13 R = -CH_2NH_2 IC_{50} = 116 nM

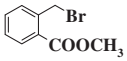
6 R = -CH=N-$(CH_2)_{n-1}$-CH_3

7 R = -CH_2-NH-$(CH_2)_{n-1}$-CH_3

8 R = -CH_2-NH-$(CH_2)_n$-NH_2

Fig. 2. Structures of NOP receptor ligands and synthesized compounds.

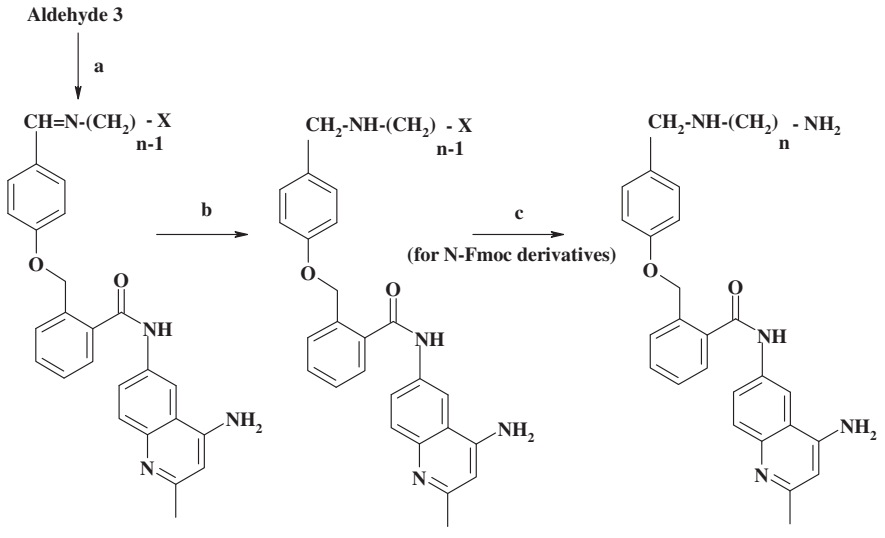


a:  **K_2CO_3 / DMF, 25°C; b: 16% aq. NaOH / THF / MeOH 1:1.5:1.5, 80°C; c: $(COCl)_2$, CH_2Cl_2, 25°C ;**

d: 4,6-Diamino-2-methylquinoline, Pyridine, 25°C; e: $NH_2(CH_2)nNH_2$ (n = 3 - 10) , MeOH, 25°C;

f: $NaBH_4$, MeOH, 25°C.

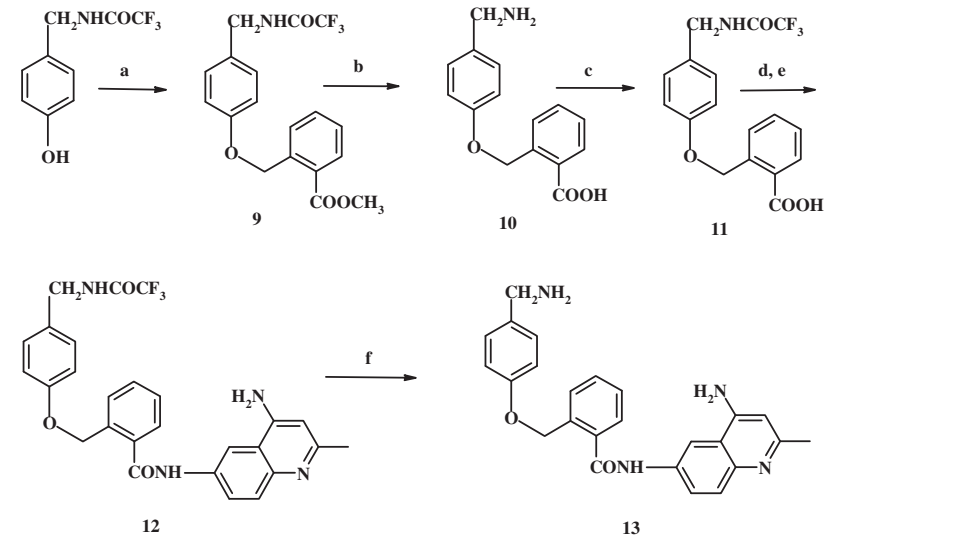
Scheme 1. Synthetical route to compounds **4** and **5**.



a: monoamine or N-Fmocdiamine(n= 3 - 10), MeOH, 25°C; b: $NaBH_4$, MeOH, 25°C;

c: 20% piperidine / DMF, 25°C.

Scheme 2. Synthetical route to compounds **6**, **7** and **8**.



Scheme 3. Synthetical route to compound **13**.

role played by the nitrogen atoms located at the extremities of the aliphatic chain. Finally *N*-(4-amino-2-methylquinolin-6-yl)-2-[(4-aminomethyl)phenoxymethyl]benzamide **13** (Fig. 2) was synthesized to evaluate the change of pharmacological properties of JTC-801 due to the substitution of the 4-ethyl group with an aminomethyl moiety.

2. Chemistry

The synthetic pathway to the new compounds is outlined in Schemes 1 and 2.

4-Hydroxybenzaldehyde was allowed to react with methyl 2-(bromomethyl)benzoate [3] and potassium carbonate to give methyl

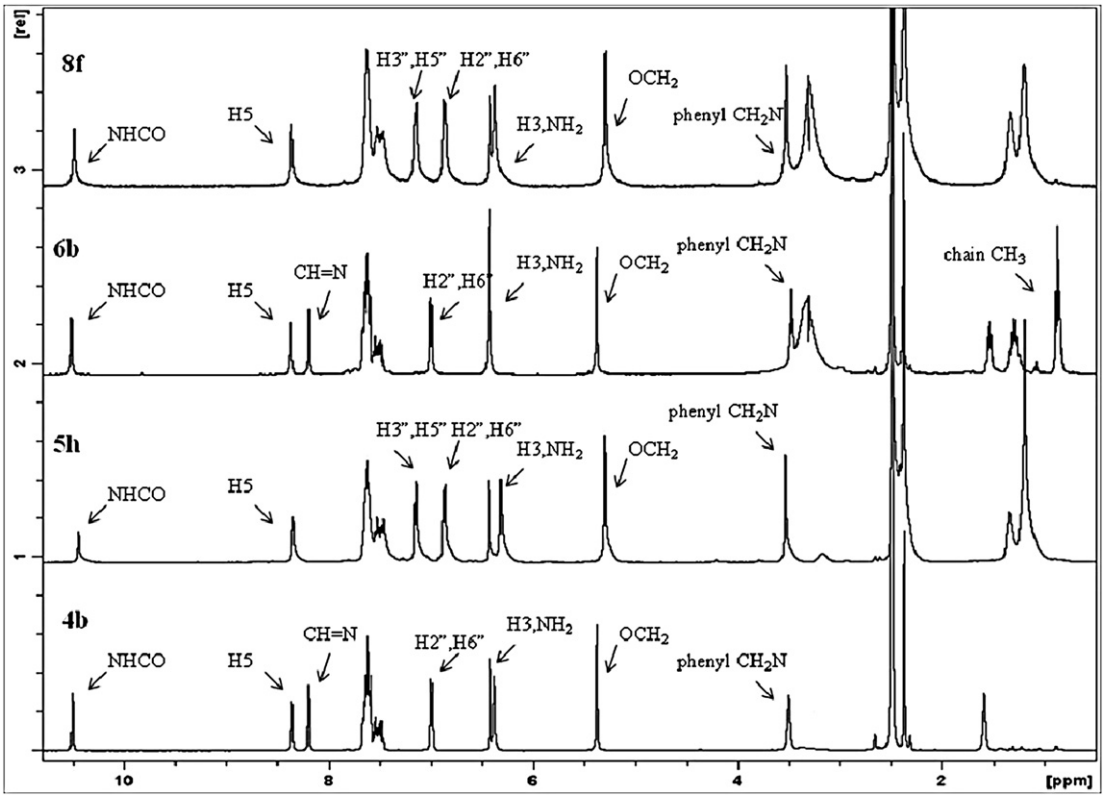
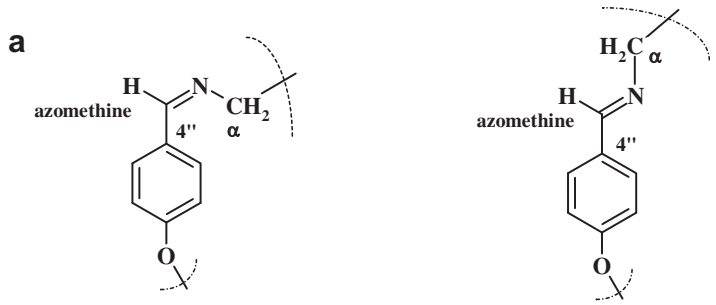
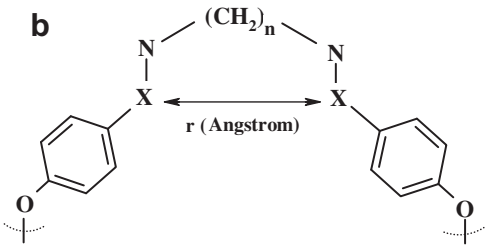


Fig. 3. ^{1}H 1D NMR spectra of the synthesized compounds. **4b** is representative of the di-iminic derivatives, **5h** is representative of the di-aminic derivatives, **6b** is representative of the alkyliminic derivatives and **8f** is representative of the aminoalkylaminic derivatives. Typical feature of compounds **4b** and **6b** is the iminic CH═ proton at 8.15 ppm. In the spectra of **4b** and **6b** the phenolic H3″,H5″ merge with the toluic protons at about 7.64 ppm, while in the spectra of **5h** and **8f** phenolic H3″,H5″ are shielded to 7.20 ppm.





	X	n	r(Å)
4f	-CH=	8	12.2
5c	$-CH_2$	5	9.8
5g	$-CH_2$	9	14.7

Fig. 4. Panel a: Azomethines **4** *cis/trans* configuration. Panel b: Distance between carbon atoms substituting the 4″ positions of the pharmacophore units in the dimeric compounds **4f**, **5c** and **5g**. The distances determined by Molecular Modelling Chem-X software were calculated for ligands at the minimum energy conformation.

2-(4-formylphenoxymethyl)benzoate **1**. The 2-(4-formylphenoxymethyl)benzoic acid **2**, obtained by alkaline hydrolysis of **1**, was treated with oxalyl chloride. The obtained acyl chloride and 4,6-diamino-2-methylquinoline [5] gave 4-amino-6-[[2-(4-formylphenoxymethyl) benzoyl]amino]-2-methylquinoline **3**. The formyl derivative **3** was the starting material for the preparation of the insaturated compounds **4** (Scheme 1) and **6** (Scheme 2) by reaction with diamines and monoalkylamines in methanol. The very slow formation of the Schiff bases **4** and **6**, as well as the previous reaction affording compound **3** were a typical example of "lazy chemistry". Reduction of azomethines **4** and **6** with sodium borohydride gave the final bivalent and univalent derivatives **5** (Scheme 1) and **7** (Scheme 2).

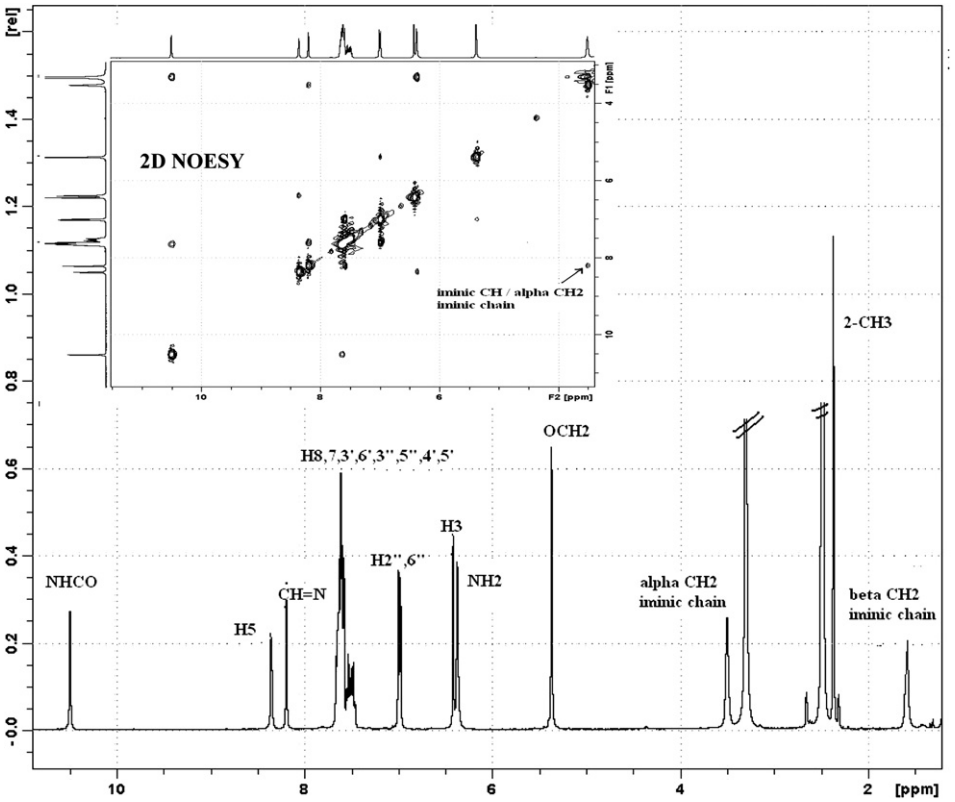


Fig. 5. ^{1}H-1D NMR spectrum of compound **4b**. 2D NOESY is inserted in the 1D spectrum. Both spectra were acquired in DMSO-d6. In the NOESY the cross peak between the azomethine proton and the alpha methylene of the amine chain is evidenced attesting the *trans* configuration of **4b**.

Aminoalkyl monomers **8a**–**h** were prepared by reaction of aldehyde **3** and N-Fmoc diamines following the procedure previously described for the monomers **7** (Scheme 2). Final Fmoc group cleavage mixture gave the expected compounds **8**.

The synthesis of compound **13** is outlined in Scheme 3. The starting product, 2,2,2-trifluoro-N-[(4-hydroxyphenyl)methyl] acetamide [15], was O-alkylated with methyl 2-(bromomethyl) benzoate to give **9**, which in turn, by alkaline hydrolysis to the aminoacid **10** and reprotection of the amine group with trifluoroacetic anhydride, gave **11**. Coupling of the acyl chloride derived from the latter with 4,6-diamino-2-methylquinoline gave **12**, which eventually, by removal of the trifluoroacetyl group with methanolic ammonia, gave the target compound **13**.

The structure of all compounds was confirmed by mono and bidimensional ^{1}H NMR experiments and MS spectrometry. Some representative ^{1}H NMR monodimensional spectra have been reported (Fig. 3).

Cis/*trans* configuration of imines **4** (Fig. 4a) was established by 2D NOESY NMR experiment performed on compound **4b** (Fig. 5). NOE was observed between the azomethinic proton and the N-α methylene group of the aminic spacer. Therefore, results supported the hypothesis of a short spatial distance between these groups, which is in agreement with a *trans* configuration. Accordingly, no NOE cross peaks were detected between the N-α methylene group and the phenolic protons that should be spatially close in the *cis* configuration.

3. Pharmacology

The binding affinities of the synthesized compounds were measured by employing [^{125}I]-nociceptin as radiotracer [16]. The most active compounds were also tested on δ, κ and μ receptors to estimate their selectivity towards all the opioid receptor subtypes. In such experiments [^{3}H]-naltrindole for δ, and [^{3}H]-diprenorphine for κ and μ were used as radioligands [17,18]. The results are shown in Table 1 and Table 2.

The biological efficacy of the compounds was evaluated as their ability to enhance the binding of [^{35}S]GTPγS in the presence of a fixed or varied concentration of GDP, using membranes isolated from cells transfected with NOP receptors. The membranes were prepared from a cell line expressing a tandem fusion protein between the NOP receptor and the α subunit of G_o. As previously reported [16], the forced 1:1 stoichiometry of expression of receptor and Gα subunit in such system greatly enhances the GTPγS response and allows detecting even small levels of ligand efficacy.

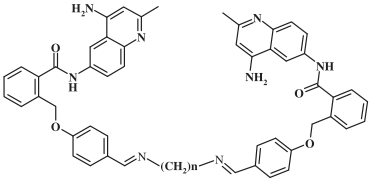
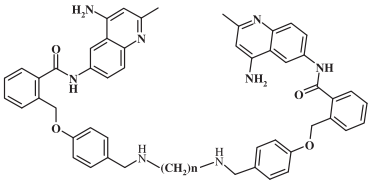
4. Results and discussion

4.1. Affinity for NOP receptor

Affinity data of all the synthesized compounds were reported in Tables 1 and 2 and an overview of the affinity − structure relationship of **4** and **5** bivalent derivatives was depicted in Fig. 6. Generally, compounds **4** showed a decrease in affinity in respect to the lead compound JTC-801. This trend was more pronounced in the case of long chain spacers and may be explained by the presence of the *trans* azomethine double bond that conferred a rigid distended shape to these molecules (Fig. 4a). The presence of the double bond proved to be less detrimental for binding for the smaller molecules **4a**–**c**. Elongation of the spacer up to 10 methylene units led to a severe loss in affinity in compounds **4d**–**h**. The lowest affinity was reached by **4f** ($n = 8$, $IC_{50} = 113.6$ nM), which is characterized by a distance of 12 Å between the methinic carbons of the pharmacophoric units (Fig. 4b). Results suggested that the interaction with the protein could be influenced by the orientation and flexibility of molecules. In agreement with this hypothesis, an opposite trend was detected for the more flexible diamines **5** whose affinity increased with chain elongation. Compounds **5** bearing the shorter spacers interacted with the protein more weakly than the corresponding unsaturated compounds **4**. Compound **5c** ($n = 5$, $IC_{50} = 76.2$ nM), for which a distance of about 10 Å (Fig. 4b) was calculated, was the less active compound in this series (Table 1). Aliphatic chain elongation from 7 to 10 methylene units, potentially extending to a distance of 16.3 Å, led to a gradual recovery of affinity (Fig. 6). Affinity reached its best value in derivative **5g** ($n = 9$, $IC_{50} = 28.1$ nM, distance 14.7 Å).

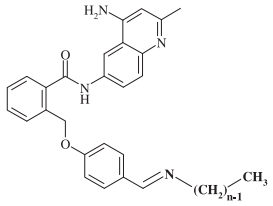
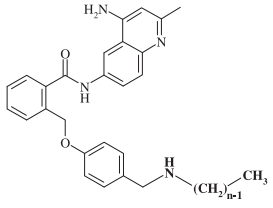
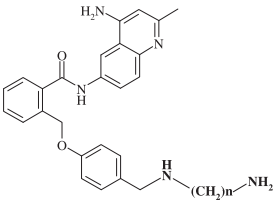
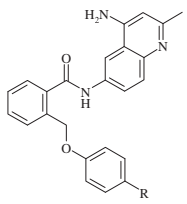
The removal of one pharmacophore unit in compounds **4** caused a marked decrease in affinity. So the univalent alkylimino compounds **6** showed IC_{50} values in a range of about 100–300 nM. The aliphatic tail played a negative role in the ligand–protein

Table 1
Binding affinity of dimeric compounds **4** and **5**.

Compound		Compound	Affinity			Selectivity	
Structure	Linker		IC_{50}(nM) ± SD				
	n		NOP	κ	μ	$IC_{50}κ/IC_{50}$NOP	$IC_{50}μ/IC_{50}$NOP
	3	**4a**	48.0 ± 11.0	–	–	–	–
	4	**4b**	35.6 ± 4.7	–	–	–	–
	5	**4c**	50.9 ± 10.7	–	–	–	–
	6	**4d**	83.3 ± 6.1	–	–	–	–
	7	**4e**	63.1 ± 16.0	–	–	–	–
	8	**4f**	113.6 ± 10.3	–	–	–	–
	9	**4g**	82.7 ± 2.8	–	–	–	–
	10	**4h**	82.1 ± 3.0	–	–	–	–
	3	**5a**	76.5 ± 15.9	5475 ± 70	468 ± 10	177	15
	4	**5b**	56.6 ± 4.7	1091 ± 15	1590 ± 25	27	39
	5	**5c**	76.2 ± 2.9	1496 ± 20	2505 ± 55	28	46
	6	**5d**	71.7 ± 14.5	1347 ± 18	1390 ± 17	30	31
	7	**5e**	30.1 ± 1.1	1023 ± 12	1038 ± 15	33	34
	8	**5f**	31.6 ± 2.3	1269 ± 63	1900 ± 59	37	56
	9	**5g**	28.1 ± 1.9	1049 ± 16	1160 ± 64	36	40
	10	**5h**	32.7 ± 7.1	900 ± 12	1320 ± 16	31	45
JTC-801			30.0 ± 1.3	7512 ± 80	1507 ± 19	250	50

Radioligands: [^{125}I]-nociceptin for NOP, [^{3}H]-naltrindole for δ and [^{3}H]-diprenorphine for κ and μ receptors. Data are given as mean ± SD ($n = 3$).

Table 2
Binding affinity of monomeric compounds **6**, **7**, **8** and of compounds **3** and **13**.

Compound			Affinity			Selectivity	
Structure	Substituent	Compound	IC_{50}(nM) ± SD				
	n		NOP	κ	μ	$IC_{50}κ/IC_{50}$NOP	$IC_{50}μ/IC_{50}$NOP
	3	**6a**	98.9 ± 5.8	–	–	–	–
	4	**6b**	308.9 ± 18.5	–	–	–	–
	5	**6c**	186.2 ± 71.4	–	–	–	–
	6	**6d**	208.6 ± 26.4	–	–	–	–
	7	**6e**	141.9 ± 39.8	–	–	–	–
	8	**6f**	146.5 ± 18.2	–	–	–	–
	9	**6g**	154.5 ± 2.5	–	–	–	–
	10	**6h**	184.5 ± 91.2	–	–	–	–
	3	**7a**	83.0 ± 6.0	3079 ± 87	3345 ± 94	45	49
	4	**7b**	1115.9 ± 32.6	5924 ± 78	5114 ± 83	10	8.6
	5	**7c**	731.6 ± 32.2	3669 ± 109	4453 ± 67	13	16
	6	**7d**	1217.8 ± 82.7	1157 ± 63	2593 ± 51	1.2	2.6
	7	**7e**	667.5 ± 32.0	1850 ± 24	1977 ± 25	6.2	6.6
	8	**7f**	482.9 ± 57.8	4955 ± 74	4339 ± 67	15	13
	9	**7g**	304.6 ± 28.3	2565 ± 58	2171 ± 55	13	11
	10	**7h**	404.7 ± 37.7	4348 ± 61	2755 ± 62	15	9.6
	3	**8a**	159.6 ± 2.6	1649 ± 21	1402 ± 19	14	12
	4	**8b**	799.8 ± 67.1	4257 ± 67	5333 ± 76	7.2	9.0
	5	**8c**	206.9 ± 42.9	2155 ± 53	4885 ± 78	18	41
	6	**8d**	1106.7 ± 34.8	1222 ± 57	5999 ± 80	1.5	7.4
	7	**8e**	246.4 ± 34.8	998 ± 10	9978 ± 143	5.7	57
	8	**8f**	144.0 ± 6.4	872 ± 17	325 ± 11	8.6	3.2
	9	**8g**	211.6 ± 3.4	6177 ± 72	7071 ± 124	40	46
	10	**8h**	152.9 ± 17.3	9103 ± 98	1401 ± 18	86	13
	R = CH_2NH_2	**13**	115.7 ± 10.4	–	–	–	
	R = CHO	**3**	207.3 ± 3.3	–	–	–	
	R = CH_2CH_3	JTC-801	30.0 ± 1.3	7512 ± 80	1507 ± 19	250	50

Radioligands: [^{125}I]-nociceptin for NOP, [^{3}H]-naltrindole for δ and [^{3}H]-diprenorphine for κ and μ receptors. Data are given as mean ± SD ($n = 3$).

interaction and the chain length modulated the effect. The best affinity was reached in derivative **6a** ($n = 3$, $IC_{50} = 98.9$ nM) and the lowest one in derivative **6b** ($n = 4$, $IC_{50} = 308.9$ nM). In the insaturated compounds **6** the detrimental effect of the aliphatic chain was weaker than that recorded in the saturated congeners **7**. Probably, in derivatives **6** the aliphatic substituent was oriented by the *trans* iminic function in such a way as to be tolerated by the receptor region surrounding the recognition site, somehow allowing a moderate interaction.

Binding experiments on univalent derivatives **7**, **8** and **13** revealed a general loss in affinity, both in respect to the corresponding dimers **5** and in respect to the lead compound JTC-801. The amine function resulted unsuitable for binding and compound **13** showed a diminished affinity ($IC_{50} = 115.7$ nM). In compounds **7**, the linker aliphatic chain exerted a further detrimental effect on affinity. This effect was more pronounced in compounds **7b**–**e** bearing chains from 4 to 8 carbon atoms. Compound **7a** ($n = 3$, $IC_{50} = 83.0$ nM) showed a moderate loss in affinity as compared with JTC-801, indicating that a short chain could be tolerated in the interaction with the peculiar site of 4-aminoquinoline derived pharmacophores. Increase of the lipophilic tail strongly disturbed interaction, probably addressing the molecule far away from the pharmacophore site. Further chain elongation led to a partial recovery of affinity in compounds **7f**–**h**. The highest affinity was displayed by compound **7g** ($n = 9$, $IC_{50} = 304.6$ nM). This behaviour suggested that longer chains could reach the protein in a lipophilic domain somehow in proximity of the pharmacophore site. Diamines **8** showed a general improved affinity in respect to monomers **7**, while maintaining the same trend in relation to the chain length. The effect could be ascribed to the presence of the primary amine function at the end of the chain. Compounds **8f**–**h** showed the best affinity in the series. This behaviour seemed to suggest the presence of an acid lipophilic environment to which aminoalkyl chains of proper length could be docked thus modulating the ligand affinity.

4.2. Affinity for δ, κ, μ receptors

Compounds **5**, **7** and **8** were tested to determine their affinity for the δ, κ, μ receptors. Data reported in Tables 1 and 2 were compared with the values detected for JTC-801 taken as a reference. All compounds showed no affinity for δ receptor and interacted weakly with κ and μ, without revealing a substantial preference. The presence of the second pharmacophore unit in the bivalent

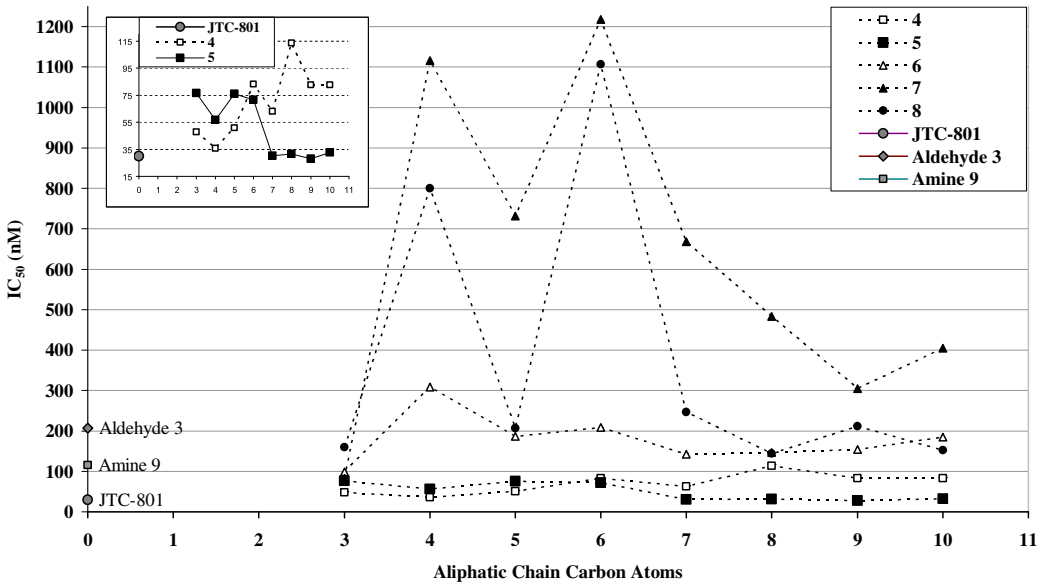


Fig. 6. Structure- NOP Receptor affinity relationship for compounds **4**, **5**, **6**, **7**, **8**.

derivatives **5** seemed to play a positive role in the interaction, mainly when the target was the κ receptor. This was the case of ligand **5h** that demonstrated eight times higher affinity than JTC-801 with $IC_{50} = 900 \pm 12$ nM. Also monomers **7** and **8** compared to JTC-801 exhibited a general enhanced affinity for the κ receptor, reaching the best value of $IC_{50} = 872 \pm 17$ nM in compound **8f**. Difference in affinity for κ receptor between compounds **5** and their related **7** and **8** was more pronounced for compounds bringing aliphatic chain with four, nine and ten methylene units. Thus, **5h** had 5- and 10-fold higher affinity than respectively **7h** and **8h**.

In the interaction with μ receptor, a moderate difference in affinity between bivalent **5** and the corresponding **7** was detected. All the bifunctional compounds showed somewhat higher affinity (about 2-fold) than the corresponding univalent congeners. The only exception was represented by **5a** that exhibited a 7-fold higher affinity than the corresponding monomer **7a**. The difference between the affinities of compounds **5** and their related ω-aminoalkyl monomers **8** were accentuated in compounds with seven and nine methylene units. Thus, the derivatives **5e** and **5g** showed affinity 10- and 6-fold higher than **8e** and **8g** respectively.

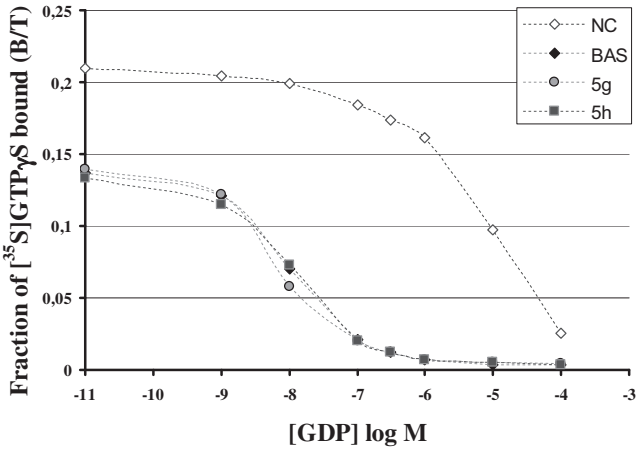


Fig. 7. Effects of compounds **5g** and **5h** on GTPγS binding in comparison with nociceptin NC The binding of [^{35}S]GTPγS was measured at increasing GDP concentrations as indicated on *x*-axis, in the absence (BAS) or in the presence of the ligands. The compounds exhibit lack of stimulation at all concentrations of GDP.

Exceptions to this trend were represented by compounds **8f** and **8h** that exhibited a better affinity at μ receptor than the corresponding dimers **5f** and **5h**.

Selectivity of these NOPr ligands towards κ and μ receptors was reported in Table 1 and Table 2 as κ/NOP and μ/NOP IC_{50} ratio.

As compared to JTC-801, compounds **5** showed a moderate loss in selectivity vs μ receptor. That behaviour was not confirmed for κ receptor to which compounds **5** bound more strongly than JTC-801. The alkyl derivatives **7** and **8** showed the same trend in selectivity of bivalent congeners and showed a significant decrease in the κ/NOP IC_{50} ratio. At last the spacer length did not substantially influence selectivity of all the tested compounds.

4.3. Activity at NOP receptor

All the compounds evaluated for their ability to enhance the binding of [^{35}S]GTPγS in the presence of 300 nM GDP, showed antagonistic properties maintaining the pharmacological behaviour of the originator JTC-801. None of the structural modifications introduced any agonistic property in the JTC-801 pharmacophore, despite the wide differences in binding affinities. A more in-depth study of the compounds with the highest binding affinity (**5g**,**h**) demonstrated that the lack of agonist activity was maintained at all GDP concentrations (Fig. 7).

5. Conclusions

Bivalent structures **5** did not exhibit affinity properties substantially from that of JTC-801. At a first glance their behaviour indicated that the second pharmacophore unit was not involved in the interaction. Nevertheless, the study pointed out that the adverse effect of spacer detected in compounds **7**, **8** could be neutralized in compounds **5** by the second pharmacophore unit. Recovery of affinity measured in dimers **5** for a chain length ranging from 10 to 14 Å (**5e**–**h**) might be explained by the presence of a further recognition site in the receptorial architecture. However on this basis it was not possible to establish whether or not this second site could belong to the same receptorial entity.

The pharmacological behaviour of our ligands **5**, compared with that exhibited by the spiropiperidine-based bivalent agonists [13], seemed to substantiate the hypothesis that JTC-801 and its

structurally related antagonists bound to a different site in respect to the competitive agonists. It is known that the NOP orthosteric binding site required very strict structural features of the ligands [19]. Therefore, the presence of a second pharmacophoric unit in the Clark compound caused a drop in affinity (Fig. 1). This behaviour could be ascribed either to an unsuitable molecular size of the ligand, if it binds to a monomeric receptor, or to an unsuitable length of the spacer considering binding to a dimeric receptor. An opposite behaviour occurred for the antagonists here described. Bivalent ligands **5** maintain the JTC-801 affinity, despite the adverse effect of the linker chain. Moreover, they display the highest affinity for linker chains of at least eight methylene units. This may be explained hypothesizing either the unlikely presence of an allosteric site able to accommodate huge molecules as ligands **5**, or supposing the existence of two separated synergic sites probably belonging to different units in a receptor dimeric assembly. A final consideration concerns the increase of affinities in monoalkylamino monomers **8** in relation to the linker chain elongation. This finding indicated that receptor interaction could be assisted by a lipophilic acidic environment. More experiments are in program to further elucidate this point.

In conclusion, the study here reported could open new perspectives and stimulate further investigation for the synthesis of ligands for the allosteric site of NOPr in its monomeric or possible dimeric form.

6. Experimental

6.1. Chemistry

Melting points were determined on a Köfler hot stage apparatus and are uncorrected.

Column chromatographic separations were accomplished on Merck aluminium oxide 90. The purity of each compound was checked on Merck aluminium oxide 60 F_{254} plates and spots were located by UV light. Anhydrous sodium sulfate was used to dry organic solutions. Elemental analyses indicated by the symbols of the elements were performed by a Carlo Erba EA 1110 instrument and were within ±0.4% of the theoretical values. The purity of described compounds determined by microanalysis resulted ≥95%.

^{1}H NMR spectra were acquired on a broad band Bruker Avance 400 equipped with temperature controller and z gradient coils. During acquisition the temperature was maintained at 298 K. Chemical shifts were reported in ppm (δ) and standard abbreviations were used (a = apparent; b = broad; d = doublet; dd = doublet of doublets; m = multiplet; q = quadruplet; s = singlet; t = triplet; u = unresolved).

2D TOCSY experiments were collected for 32 scans at 2048 × 512 data points, for a mixing time of 100 ms, spectra were processed by sine bell function and zero-filled to 1024 in the t1 dimension.

2D NOESY experiments were collected for 32 scans at 2048 × 512 data points, for mixing time of 300 ms, 500 ms and 1 s, spectra were processed by sine bell function and zero-filled to 1024 in the t1 dimension.

2D NOESY NMR experiment aimed to elucidate *cis*/*trans* configuration of imines **4** was established by at mixing times corresponding to the T_1 relaxation times of the molecular fragments composing **4**.

High Resolution Mass spectra were recorded on a Micromass (Now Waters) Q-TOF Micro Spectrometer with ESI (+) source.

Methyl 2-(bromomethyl)benzoate [3] and 4,6-diamino-2-methylquinoline [5] were obtained according literature methods. 2,2,2-Trifluoro-N-[(4-hydroxyphenyl)methyl]acetamide was prepared as described in the patent literature [15].

6.1.1. Fmoc diamines

N-Fmoc-1,3-propane, N-Fmoc-1,4-butane N-Fmoc-1,5-pentane and N-Fmoc-1,6-hexane diamines were purchased by Fluka as hydrobromide salts. The other N-Fmoc diamines were prepared as follows: to a diluted solution of diamine (Aldrich) (7.6 mmol) in anhydrous tetrahydrofuran (50 mL), a solution of Fmoc chloride (Sigma) (0.5 g, 1.9 mmol) in anhydrous tetrahydrofuran (50 mL) was slowly added at 0 °C for 3 h. The suspension was allowed to react overnight at room temperature, and then the solvent was evaporated in vacuo. N-Fmoc diamine was extracted with ethyl ether. Evaporation of the solvent gave the expected products as viscous oils. The compounds, whose structures were confirmed by ^{1}H NMR, were used without further purification (90% purity determined by microanalysis).

6.1.2. Methyl 2-(4-formylphenoxymethyl)benzoate (*1*)

A mixture of 4-hydroxybenzaldehyde (3.7 g, 30 mmol), methyl 2-(bromomethyl)benzoate (6.9 g, 30 mmol) and anhydrous K_2CO_3 (20.7 g, 150 mmol) in anhydrous DMF (100 mL) was kept under stirring at 30 °C for 4 h, then ice water was added. The resulting white precipitate was collected by filtration, washed with water and dried under vacuum to obtain **1** (6.9 g, 85%); mp 76–78 °C; ^{1}H NMR (DMSO-d_6) δ: 9.87 (s, 1H, 4′-CHO), 7.92 (d, 1H, H-6), 7.87 (d, 2H, H-3′,5′), 7.65 (sharp m, 2H, H-3,4), 7.49 (t, 1H, H-5), 7.17 (d, 2H, H-2′,6′), 5.53 (s, 2H, OCH_2), 3.80 (s, 3H, OCH_3). MS (ESI) *m*/*z*: 271 $(M + H)^+$. Anal. Calcd. for $C_{16}H_{14}O_4$: C, 71.09; H, 5.22. Found: C, 71.10; H, 5.19.

6.1.3. 2-(4-Formylphenoxymethyl)benzoic acid (*2*)

A solution of **1** (5.7 g, 21 mmol) in tetrahydrofuran (18 mL), methanol (18 mL) and 16% aqueous NaOH (12 mL) was heated at 80 °C under stirring for 45 min. After cooling the solution was acidified with diluted HCl and the white precipitate **2** was collected by filtration, washed with water and dried in an oven at 50 °C under vacuum. Yield 4.1 g, 76%; mp 165–167 °C ^{1}H NMR (DMSO-d_6) δ: 9.88 (s, 1H, 4′-CHO), 7.96 (d, 1H, H-6), 7.89 (d, 2H, H-3′,5′), 7.62 (sharp m, 2H, H-3,4), 7.48 (t, 1H, H-5), 7.18 (d, 2H, H-2′,6′), 5.58 (s, 2H, OCH_2). MS (ESI) *m*/*z*: 279 $(M + Na)^+$. Anal. Calcd. for $C_{15}H_{12}O_4$: C, 70.29; H, 4.72. Found: C, 70.38; H, 4.79.

6.1.4. 4-Amino-6-[[2-(4-formylphenoxymethyl)benzoyl]amino]-2-methylquinoline (*3*)

To a solution of **2** (2.0 g, 7.8 mmol) in anhydrous CH_2Cl_2 (80 mL), oxalyl chloride (1.4 mL, 16 mmol) was added with a few drops of DMF. The resulting mixture was stirred for 48 h at room temperature. Evaporation of the solvent gave 2-(4-formylphenoxymethyl) benzoyl chloride as a clear syrup which was dissolved in anhydrous pyridine (100 mL). To the solution cooled in an ice bath 4,6-diamino-2-methylquinoline (1.3 g, 7.5 mmol) was added under stirring. The mixture was allowed to react for weekend at 25 °C, then the solution was decanted from the undissolved precipitate and evaporated in vacuo. The residue was suspended in a diluted NaOH solution and thoroughly extracted with ethyl acetate. The organic layer was washed with brine and concentrated in vacuo. The residue was chromatographed on an aluminium oxide column eluting with ethyl acetate/methanol 9:1 mixture. The obtained compound **3** was used without further purification. Yield 0.71 g, 22%. An analytical sample was obtained by crystallization from ethyl acetate: mp 125–128 °C ^{1}H NMR (DMSO-d_6) δ: 10.54 (s, 1H, NH), 9.82 (s, 1H, 4″-CHO), 8.36 (s, 1H, H-5), 7.81 (d, 2H, H-3″,5″), 7.63 (sharp m, 5H, H-7,8, H-3′,4′,6′), 7.53 (t, 1H, H-5′), 7.15 (d, 2H, H-2″,6″), 6.47 (bs, 2H, 4-NH_2), 6.43 (s, 1H, H-3), 5.46 (s, 2H, OCH_2), 2.38 (s, 3H, 2-CH_3). MS (ESI) *m*/*z*: 412 $(M + H)^+$. Anal. Calcd. for $C_{25}H_{21}N_3O_3$: C, 72.96; H, 5.15; N, 10.22. Found: C, 72.85; H, 5.11; N, 10.20.

*6.1.5. General procedure for the preparation of bivalent ligands of N-(4-amino-2-methylquinolin-6-yl)-2-(4-aminomethylphenoxymethyl)benzamide (**5**)*

(a) Preparation of azomethines **4**. To a concentrated solution of **3** (0.3 g, 0.73 mmol) in methanol (3 mL), the appropriate diamine (0.36 mmol) was added at room temperature. The solution was stirred and after a while a precipitate separated. The suspension was kept under stirring for the weekend, then the solid Schiff base **4** was filtered, washed with methanol and ether and dried in vacuo.

(b) Reduction of **4**. Compounds **4** (0.1 g) suspended in methanol (5 mL) at room temperature were reduced by sodium borohydride (0.5 g) under stirring. After 2 h the resulting solution was concentrated in vacuo and the residue treated with water. The solid compounds **5** that separated were filtered and rinsed thoroughly with water and ethyl ether.

*6.1.5.1. (E,E)-N,N′-bis[[4-[[2-[[(4-amino-2-methylquinolin-6-yl)amino]carbonyl]phenyl]methoxy]phenyl]methylene]-1,3-propanediamine (**4a**).* Yield 50%, mp 165–167 °C ^{1}H NMR (DMSO-d_6) δ: 10.51 (s, 2H, NH), 8.36 (s, 2H, H-5), 8.20 (s, 2H, CH=N), 7.63 (m, 12 H, H-8, H-7, H-3′, H-6′, H-3″,5″), 7.54 (t, 2H, H-4′), 7.49 (t, 2H, H-5′), 7.00 (d, 4H, H-2″,6″), 6.43 (s, 2H, H-3), 6.39 (s, 4H, 4-NH_2), 5.38 (s, 4H, OCH_2), 3.54 (t, 4H, chain N–CH_2), 2.38 (s, 6H, 2-CH_3), 1.87 (quintuplet, 2H, chain central –CH_2–). MS (ESI) *m*/*z*: 861 $(M + H)^+$. Anal. Calcd. for $C_{53}H_{48}N_8O_4$: C, 73.92; H, 5.62; N, 13.02. Found: C, 73.96; H, 5.58; N, 13.12.

*6.1.5.2. (E,E)-N,N′-bis[[4-[[2-[[(4-amino-2-methylquinolin-6-yl)amino]carbonyl]phenyl]methoxy]phenyl]methylene]-1,4-butanediamine (**4b**).* Yield 41%, mp 153–155 °C ^{1}H NMR (DMSO-d_6) δ: 10.51 (s, 2H, NH), 8.36 (s, 2H, H-5), 8.20 (s, 2H, CH=N), 7.62 (m, 12 H, H-8, H-7, H-3′, H-6′, H-3″,5″), 7.54 (t, 2H, H-4′), 7.51 (t, 2H, H-5′), 6.99 (d, 4H, H-2″,6″), 6.42 (s, 2H, H-3), 6.39 (s, 4H, 4-NH_2), 5.37 (s, 4H, OCH_2), 3.50 (t, 4H, chain N–CH_2), 2.37 (s, 6H, 2-CH_3), 1.59 (m, 4H, chain central –$(CH_2)_2$–). MS (ESI) *m*/*z*: 875 $(M + H)^+$. Anal. Calcd. for $C_{54}H_{50}N_8O_4$: C, 74.11; H, 5.76; N, 12.81. Found: C, 74.15; H, 5.78; N, 12.84.

*6.1.5.3. (E,E)-N,N′-bis[[4-[[2-[[(4-amino-2-methylquinolin-6-yl)amino]carbonyl]phenyl]methoxy]phenyl]methylene]-1,5-pentanediamine (**4c**).* Yield 55%, mp 152–153 °C ^{1}H NMR (DMSO-d_6) δ: 10.52 (s, 2H, NH), 8.36 (s, 2H, H-5), 8.19 (s, 2H, CH=N), 7.63 (m, 12 H, H-8, H-7, H-3′, H-6′, H-3″,5″), 7.54 (t, 2H, H-4′), 7.50 (t, 2H, H-5′), 6.99 (d, 4H, H-2″,6″), 6.42 (s, 2H, H-3), 6.39 (s, 4H, 4-NH_2), 5.38 (s, 4H, OCH_2), 3.50 (t, 4H, chain N–CH_2), 2.37 (s, 6H, 2-CH_3), 1.58 (m, 4H, chain –CH_2–), 1.31 (m, 2H, chain central –CH_2–). MS (ESI) *m*/*z*: 889 $(M + H)^+$. Anal. Calcd. for $C_{55}H_{52}N_8O_4$: C, 74.29; H, 5.90; N, 12.61. Found: C, 74.31; H, 5.94; N, 12.60.

*6.1.5.4. (E,E)-N,N′-bis[[4-[[2-[[(4-amino-2-methylquinolin-6-yl)amino]carbonyl]phenyl]methoxy]phenyl]methylene]-1,6-hexanediamine (**4d**).* Yield 43%, mp 155–157 °C ^{1}H NMR (DMSO-d_6) δ: 10.52 (s, 2H, NH), 8.38 (s, 2H, H-5), 8.20 (s, 2H, CH=N), 7.64 (m, 12 H, H-8, H-7, H-3′, H-6′, H-3″,5″), 7.56 (t, 2H, H-4′), 7.49 (t, 2H, H-5′), 7.01 (d, 4H, H-2″,6″), 6.44 (s, 2H, H-3), 6.40 (s, 4H, 4-NH_2), 5.39 (s, 4H, OCH_2), 3.48 (t, 4H, chain N–CH_2), 2.39 (s, 6H, 2-CH_3), 1.58 (m, 4H, chain –CH_2–), 1.32 (m, 4H, chain central –$(CH_2)_2$–). MS (ESI) *m*/*z*: 903 $(M + H)^+$. Anal. Calcd. for $C_{56}H_{54}N_8O_4$: C, 74.47; H, 6.03; N, 12.41. Found: C, 74.48; H, 6.05; N, 12.43.

*6.1.5.5. (E,E)-N,N′-bis[[4-[[2-[[(4-amino-2-methylquinolin-6-yl)amino]carbonyl]phenyl]methoxy]phenyl]methylene]-1,7-heptanediamine (**4e**).* Yield 38%, mp 120–122 °C ^{1}H NMR (DMSO-d_6) δ: 10.55 (s, 2H, NH), 8.39 (s, 2H, H-5), 8.20 (s, 2H, CH=N), 7.64 (m, 12 H, H-8, H-7, H-3′, H-6′, H-3″,5″), 7.54 (t, 2H, H-4′), 7.50 (t, 2H, H-5′), 7.01 (d, 4H, H-2″,6″), 6.44 (s, 2H, H-3), 6.41 (s, 4H, 4-NH_2), 5.39 (s, 4H, OCH_2), 3.49 (t, 4H, chain N–CH_2), 2.39 (s, 6H, 2-CH_3), 1.57 (m, 4H, chain –CH_2–), 1.29 (bm, 6H, chain –CH_2–). MS (ESI) *m*/*z*: 917 $(M + H)^+$. Anal. Calcd. for $C_{57}H_{56}N_8O_4$: C, 74.64; H, 6.16; N, 12.22. Found: C, 74.63; H, 6.15; N, 12.20.

*6.1.5.6. (E,E)-N,N′-bis[[4-[[2-[[(4-amino-2-methylquinolin-6-yl)amino]carbonyl]phenyl]methoxy]phenyl]methylene]-1,8-octanediamine (**4f**).* Yield 42%, mp 118–120 °C ^{1}H NMR (DMSO-d_6) δ: 10.50 (s, 2H, NH), 8.36 (s, 2H, H-5), 8.18 (s, 2H, CH=N), 7.62 (m, 12 H, H-8, H-7, H-3′, H-6′, H-3″,5″), 7.53 (t, 2H, H-4′), 7.50 (t, 2H, H-5′), 7.00 (d, 4H, H-2″,6″), 6.42 (s, 2H, H-3), 6.38 (s, 4H, 4-NH_2), 5.37 (s, 4H, OCH_2), 3.46 (t, 4H, chain N–CH_2), 2.37 (s, 6H, 2-CH_3), 1.56 (m, 4H, chain –CH_2–), 1.26 (bs, 8H, chain –CH_2–). MS (ESI) *m*/*z*: 931 $(M + H)^+$. Anal. Calcd. for $C_{58}H_{58}N_8O_4$: C, 74.80; H, 6.28; N, 12.04. Found: C, 74.82; H, 6.31; N, 12.07.

*6.1.5.7. (E,E)-N,N′-bis[[4-[[2-[[(4-amino-2-methylquinolin-6-yl)amino]carbonyl]phenyl]methoxy]phenyl]methylene]-1,9-nonanediamine (**4g**).* Yield 58%, mp 138–140 °C ^{1}H NMR (DMSO-d_6) δ: 10.53 (s, 2H, NH), 8.39 (s, 2H, H-5), 8.20 (s, 2H, CH=N), 7.64 (m, 12 H, H-8, H-7, H-3′, H-6′, H-3″,5″), 7.54 (t, 2H, H-4′), 7.50 (t, 2H, H-5′), 7.01 (d, 4H, H-2″,6″), 6.44 (s, 2H, H-3), 6.43 (s, 4H, 4-NH_2), 5.39 (s, 4H, OCH_2), 3.47 (t, 4H, chain N–CH_2), 2.39 (s, 6H, 2-CH_3), 1.54 (m, 4H, chain –CH_2–), 1.26 (bs, 10H, chain –CH_2–). MS (ESI) *m*/*z*: 945 $(M + H)^+$. Anal. Calcd. for $C_{59}H_{60}N_8O_4$: C, 74.96; H, 6.40; N, 11.86. Found: C, 74.95; H, 6.42; N, 11.88.

*6.1.5.8. (E,E)-N,N′-bis[[4-[[2-[[(4-amino-2-methylquinolin-6-yl)amino]carbonyl]phenyl]methoxy]phenyl]methylene]-1,10-decanediamine (**4h**).* Yield 57%, mp 127–129 °C ^{1}H NMR (DMSO-d_6) δ: 10.53 (s, 2H, NH), 8.39 (s, 2H, H-5), 8.20 (s, 2H, CH=N), 7.64 (m, 12 H, H-8, H-7, H-3′, H-6′, H-3″,5″), 7.54 (t, 2H, H-4′), 7.50 (t, 2H, H-5′), 7.01 (d, 4H, H-2″,6″), 6.44 (s, 2H, H-3), 6.41 (s, 4H, 4-NH_2), 5.39 (s, 4H, OCH_2), 3.45 (t, 4H, chain N–CH_2), 2.40 (s, 6H, 2-CH_3), 1.47 (m, 4H, chain –CH_2–), 1.20 (bm, 12H, chain –CH_2–). MS (ESI) *m*/*z*: 959 $(M + H)^+$. Anal. Calcd. for $C_{60}H_{62}N_8O_4$: C, 75.12; H, 6.52, N. 11.69. Found: C, 75.16; H, 6.57; N, 11.71.

*6.1.5.9. N,N′-bis[[4-[[2-[[(4-amino-2-methylquinolin-6-yl)amino]carbonyl]phenyl]methoxy]phenyl]methyl]-1,3-propanediamine (**5a**).* Yield 92%, mp 143–145 °C ^{1}H NMR (DMSO-d_6) δ: 10.51 (s, 2H, NH), 8.37 (s, 2H, H-5), 7.63 (m, 8H, H-8, H-7, H-3′, H-6′,), 7.50 (two overlapped t, 4H, H-4′, H-5′), 7.15 (d, 4H, H-3″,5″), 6.88 (d, 4H, H-2″,6″), 6.44 (s, 2H, H-3), 6.40 (s, 4H, 4-NH_2), 5.30 (s, 4H, OCH_2), 3.53 (s, 4H, 4″-CH_2N), 2.44 (t, 4H, chain N–CH_2), 2.38 (s, 6H, 2-CH_3), 1.50 (t, 2H, chain central –CH_2). MS (ESI) *m*/*z*: 865 $(M + H)^+$. Anal. Calcd. for $C_{53}H_{52}N_8O_4$: C, 73.58; H, 6.06; N, 12.96. Found: C, 73.54; H, 6.09; N, 12.94.

*6.1.5.10. N,N′-bis[[4-[[2-[[(4-amino-2-methylquinolin-6-yl)amino]carbonyl]phenyl]methoxy]phenyl]methyl]-1,4-butanediamine (**5b**).* Yield 92%, mp 127–129 °C ^{1}H NMR (DMSO-d_6) δ: 10.50 (s, 2H, NH), 8.35 (s, 2H, H-5), 7.63 (m, 8H, H-8, H-7, H-3′, H-6′,), 7.50 (two partially overlapped t, 4H, H-4′, H-5′), 7.16 (d, 4H, H-3″,5″), 6.87 (d, 4H, H-2″,6″), 6.44 (s, 2H, H-3), 6.40 (s, 4H, 4-NH_2), 5.30 (s, 4H, OCH_2), 3.54 (s, 4H, 4″-CH_2N), 2.45 (t, 4H, chain N–CH_2), 2.38 (s, 6H, 2-CH_3), 1.51 (m, 4H, chain central –$(CH_2)_2$–). MS (ESI) *m*/*z*: 879 $(M + H)^+$. Anal. Calcd. for $C_{54}H_{54}N_8O_4$: C, 73.77; H, 6.20; N, 12.75. Found: C, 73.81; H, 6.25; N, 12.73.

*6.1.5.11. N,N′-bis[[4-[[2-[[(4-amino-2-methylquinolin-6-yl)amino]carbonyl]phenyl]methoxy]phenyl]methyl]-1,5-pentanediamine (**5c**).* Yield 95%, mp 128–130 °C ^{1}H NMR (DMSO-d_6) δ: 10.49 (s, 2H, NH), 8.36 (s, 2H, H-5), 7.63 (m, 8H, H-8, H-7, H-3′, H-6′,), 7.53 (t, 2H, H-4′), 7.49 (t, 2H, H-5′), 7.15 (d, 4H, H-3″,5″), 6.87 (d, 4H, H-2″,6″), 6.43 (s, 2H, H-3), 6.38 (s, 4H, 4-NH_2), 5.30 (s, 4H, OCH_2), 3.54 (s, 4H, 4″-CH_2N), 2.38 (bs, 10H, 2-CH_3, chain N–CH_2), 1.34 (bt, 4H, chain CH_2),

1.23 (um, 2H, chain central $-CH_2$). MS (ESI) *m/z*: 893 $(M + H)^+$. Anal. Calcd. for $C_{55}H_{56}N_8O_4$: C, 73.95; H, 6.32; N, 12.55. Found: C, 73.97; H, 6.34; N, 12.50.

*6.1.5.12. N,N′-bis[[4-[[2-[[(4-amino-2-methylquinolin-6-yl)amino] carbonyl]phenyl]methoxy]phenyl]methyl]-1,6-hexanediamine (**5d**).* Yield 95%, mp 133–135 °C ^{1}H NMR (DMSO-d_6) δ: 10.48 (s, 2H, NH), 8.36 (s, 2H, H-5), 7.63 (m, 8H, H-8, H-7, H-3′, H-6′,), 7.52 (t, 2H, H-4′), 7.47 (t, 2H, H-5′), 7.13 (d, 4H, H-3″,5″), 6.85 (d, 4H, H-2″,6″), 6.42 (s, 2H, H-3), 6.37 (s, 4H, 4-NH_2), 5.29 (s, 4H, OCH_2), 3.52 (s, 4H, 4″-CH_2N), 2.37 (bs, 10H, 2-CH_3, chain N–CH_2), 1.34 (bt, 4H, chain CH_2), 1.22 (um, 4H, chain central $-(CH_2)_2-$). MS (ESI) *m/z*: 907 $(M + H)^+$. Anal. Calcd. for $C_{56}H_{58}N_8O_4$: C, 74.13; H, 6.45; N, 12.36. Found: C, 74.17; H, 6.49; N, 12.31.

*6.1.5.13. N,N′-bis[[4-[[2-[[(4-amino-2-methylquinolin-6-yl)amino] carbonyl]phenyl]methoxy]phenyl]methyl]-1,7-heptanediamine (**5e**).* Yield 92%, mp 128–130 °C ^{1}H NMR (DMSO-d_6) δ: 10.51 (s, 2H, NH), 8.38 (s, 2H, H-5), 7.64 (m, 8H, H-8, H-7, H-3′, H-6′,), 7.52 (m, 4H, H-4′, H-5′), 7.17 (d, 4H, H-3″,5″), 6.88 (d, 4H, H-2″,6″), 6.44 (s, 2H, H-3), 6.38 (s, 4H, 4-NH_2), 5.31 (s, 4H, OCH_2), 3.54 (s, 4H, 4″-CH_2N), 2.39 (bs, 10H, 2-CH_3, chain N–CH_2), 1.36 (bt, 4H, chain CH_2), 1.21 (um, 6H, chain central $-(CH_2)_3-$). MS (ESI) *m/z*: 921 $(M + H)^+$. Anal. Calcd. for $C_{57}H_{60}N_8O_4$: C, 74.31; H, 6.57; N, 12.17. Found: C, 74.29; H, 6.55; N, 12.15.

*6.1.5.14. N,N′-bis[[4-[[2-[[(4-amino-2-methylquinolin-6-yl)amino] carbonyl]phenyl]methoxy]phenyl]methyl]-1,8-octanediamine (**5f**).* Yield 93%, mp 140–142 °C ^{1}H NMR (DMSO-d_6) δ: 10.51 (s, 2H, NH), 8.38 (s, 2H, H-5), 7.64 (m, 8H, H-8, H-7, H-3′, H-6′,), 7.52 (m, 4H, H-4′, H-5′), 7.16 (d, 4H, H-3″,5″), 6.75 (d, 4H, H-2″,6″), 6.44 (s, 2H, H-3), 6.38 (s, 4H, 4-NH_2), 5.31 (s, 4H, OCH_2), 3.54 (s, 4H, 4″-CH_2N), 2.39 (bs, 10H, 2-CH_3, chain N–CH_2), 1.35 (bt, 4H, chain CH_2), 1.21 (um, 8H, chain central $-(CH_2)_4-$). MS (ESI) *m/z*: 935$(M + H)^+$. Anal. Calcd. for $C_{58}H_{62}N_8O_4$: C, 74.48; H, 6.69; N, 11.99. Found: C, 74.50; H, 6.71; N, 11.95.

*6.1.5.15. N,N′-bis[[4-[[2-[[(4-amino-2-methylquinolin-6-yl)amino] carbonyl]phenyl]methoxy]phenyl]methyl]-1,9-nonanediamine (**5g**).* Yield 96%, mp 135–137 °C ^{1}H NMR (DMSO-d_6) δ: 10.46 (s, 2H, NH), 8.36 (s, 2H, H-5), 7.63 (m, 8H, H-8, H-7, H-3′, H-6′,), 7.54 (t, 2H, H-4′), 7.48 (t, 2H, H-5′), 7.16 (d, 4H, H-3″,5″), 6.88 (d, 4H, H-2″,6″), 6.45 (s, 2H, H-3), 6.33 (s, 4H, 4-NH_2), 5.31 (s, 4H, OCH_2), 3.55 (s, 4H, 4″-CH_2N), 2.39 (bs, 10H, 2-CH_3, chain N–CH_2), 1.36 (bt, 4H, chain CH_2), 1.21 (um, 10H, chain central $-(CH_2)_5-$). MS (ESI) *m/z*: 949 $(M + H)^+$. Anal. Calcd. for $C_{59}H_{64}N_8O_4$: C, 74.64; H, 6.80; N. 11.81. Found: C, 74.67; H, 6.83; N, 11.80.

*6.1.5.16. N,N′-bis[[4-[[2-[[(4-amino-2-methylquinolin-6-yl)amino] carbonyl]phenyl]methoxy]phenyl]methyl]-1,10-decanediamine (**5h**).* Yield 96%, mp 125–127 °C ^{1}H NMR (DMSO-d_6) δ: 10.46 (s, 2H, NH), 8.36 (s, 2H, H-5), 7.63 (m, 8H, H-8, H-7, H-3′, H-6′,), 7.54 (t, 2H, H-4′), 7.48 (t, 2H, H-5′), 7.16 (d, 4H, H-3″,5″), 6.88 (d, 4H, H-2″,6″), 6.45 (s, 2H, H-3), 6.33 (s, 4H, 4-NH_2), 5.31 (s, 4H, OCH_2), 3.55 (s, 4H, 4″-CH_2N), 2.39 (bs, 10H, 2-CH_3, chain N–CH_2), 1.35 (bt, 4H, chain CH_2), 1.21 (um, 12H, chain central $-(CH_2)_6-$). MS (ESI) *m/z*: 963 $(M + H)^+$. Anal. Calcd. for $C_{60}H_{66}N_8O_4$: C, 74.80; H, 6.91; N, 11.64. Found: C, 74.87; H, 6.95; N, 11.68.

*6.1.6. General procedure for the preparation of N-(4-amino-2-methyl quinolin-6-yl)-2-[4-(alkylaminomethyl)phenoxymethyl]benzamide (**7**)*

(a) Preparation of Azomethines **6**. To a solution of **3** (0.3 g, 0.73 mmol) in methanol (5 mL) an excess of the appropriate alkylamine (1.50 mmol) was added at room temperature. The solution was stirred overnight then the solvent was evaporated. The residue was treated thoroughly with water, filtered and dried in vacuo.

(b) Reduction of **6**. Compounds **6** (0.1 g) dissolved in methanol (5 mL) at room temperature were reduced by sodium borohydride (0.5 g) under stirring. After 2 h the resulting solution was concentrated in vacuo and the residue treated with water. The solid compounds **7** that separated were filtered, rinsed thoroughly with water and dried in vacuo.

*6.1.6.1. (E)-N-(4-amino-2-methylquinolin-6-yl)-2-[4-(propylimino-methyl)phenoxymethyl]benzamide (**6a**).* Yield 75%, mp 125–127 °C ^{1}H NMR (DMSO-d_6) δ: 10.50 (s, 1H, NH), 8.36 (s, 1H, H-5), 8.18 (s, 1H, CH=N), 7.62 (m, 6 H, H-8, H-7, H-3′, H-6′, H-3″,5″), 7.54 (t, 1H, H-4′), 7.50 (t, 1H, H-5′), 6.99 (d, 2H, H-2″,6″), 6.42 (s, 1H, H-3), 6.38 (s, 2H, 4-NH_2), 5.37 (s, 2H, OCH_2), 3.44 (t, 2H, chain N–CH_2), 2.38 (s, 3H, 2-CH_3), 1.56 (m, 2H, chain CH_2), 1.29 (t, 3H, chain CH_3). MS (ESI) *m/z*: 453 $(M + H)^+$. Anal. Calcd. for $C_{28}H_{28}N_4O_2$: C, 74.30; H, 6.24; N, 12.39. Found: C, 74.29; H, 6.21; N, 12.38.

*6.1.6.2. (E)-N-(4-amino-2-methylquinolin-6-yl)-2-[4-(butylimino-methyl)phenoxymethyl]benzamide (**6b**).* Yield 72%, mp 120–123 °C. ^{1}H NMR (DMSO-d_6) δ: 10.51 (s, 1H, NH), 8.37 (s, 1H, H-5), 8.19 (s, 1H, CH=N), 7.63 (m, 6 H, H-8, H-7, H-3′, H-6′, H-3″,5″), 7.56 (t, 1H, H-4′), 7.49 (t, 1H, H-5′), 7.00 (d, 2H, H-2″,6″), 6.40 (s, 3H, H-3, 4-NH_2), 5.38 (s, 2H, OCH_2), 3.49 (t, 2H, chain N–CH_2), 2.38 (s, 3H, 2-CH_3), 1.54 (m, 2H, chain CH_2), 1.29 (m, 2H, chain CH_2), 0.88 (t, 3H, chain CH_3). MS (ESI) *m/z*: 467 $(M + H)^+$. Anal. Calcd. for $C_{29}H_{30}N_4O_2$: C, 74.64; H, 6.48; N, 12.01. Found: C, 74.67; H, 6.51; N, 12.06.

*6.1.6.3. (E)-N-(4-amino-2-methylquinolin-6-yl)-2-[4-(pentylimi-nomethyl)phenoxymethyl]benzamide (**6c**).* Yield 77%, mp 88–90 °C ^{1}H NMR (DMSO-d_6) δ: 10.50 (s, 1H, NH), 8.37 (s, 1H, H-5), 8.19 (s, 1H, CH=N), 7.63 (m, 6 H, H-8, H-7, H-3′, H-6′, H-3″,5″), 7.56 (m, 2H, H-4′, H-5′), 7.00 (d, 2H, H-2″,6″), 6.43 (s, 1H, H-3), 6.39 (s, 2H, 4-NH_2), 5.38 (s, 2H, OCH_2), 3.47 (t, 2H, chain N–CH_2), 2.38 (s, 3H, 2-CH_3), 1.56 (m, 2H, chain CH_2), 1.25 (m, 4H, chain $-(CH_2)_2-$), 0.85 (t, 3H, chain CH_3). MS (ESI) *m/z*: 481 $(M + H)^+$. Anal. Calcd. for $C_{30}H_{32}N_4O_2$: C, 74.96; H, 6.72; N, 11.66. Found: C, 74.93; H, 6.70; N, 11.62.

*6.1.6.4. (E)-N-(4-amino-2-methylquinolin-6-yl)-2-[4-(hexylimino-methyl)phenoxymethyl]benzamide (**6d**).* Yield 73%, mp 90–92 °C ^{1}H NMR (DMSO-d_6) δ: 10.52 (s, 1H, NH), 8.38 (s, 1H, H-5), 8.21 (s, 1H, CH=N), 7.64 (m, 6 H, H-8, H-7, H-3′, H-6′, H-3″,5″), 7.54 (m, 2H, H-4′, H-5′), 7.01 (d, 2H, H-2″,6″), 6.44 (s, 1H, H-3), 6.39 (s, 2H, 4-NH_2), 5.39 (s, 2H, OCH_2), 3.49 (t, 2H, chain N–CH_2), 2.40 (s, 3H, 2-CH_3), 1.57 (m, 2H, chain CH_2), 1.27 (m, 6H, chain $-(CH_2)_3-$), 0.85 (t, 3H, chain CH_3). MS (ESI) *m/z*: 495 $(M + H)^+$. Anal. Calcd. for $C_{31}H_{34}N_4O_2$: C, 75.26; H, 6.93; N, 11.33. Found: C, 75.20; H, 6.91; N, 11.34.

*6.1.6.5. (E)-N-(4-amino-2-methylquinolin-6-yl)-2-[4-(heptylimi-nomethyl)phenoxymethyl]benzamide (**6e**).* Yield 75%, mp 87–90 °C. ^{1}H NMR (DMSO-d_6) δ: 10.53 (s, 1H, NH), 8.38 (s, 1H, H-5), 8.20 (s, 1H, CH=N), 7.64 (m, 6 H, H-8, H-7, H-3′, H-6′, H-3″,5″), 7.53 (m, 2H, H-4′, H-5′), 7.01 (d, 2H, H-2″,6″), 6.44 (s, 1H, H-3), 6.41 (s, 2H, 4-NH_2), 5.39 (s, 2H, OCH_2), 3.48 (t, 2H, chain N–CH_2), 2.39 (s, 3H, 2-CH_3), 1.58 (m, 2H, chain CH_2), 1.26 (m, 8H, chain $-(CH_2)_4-$), 0.85 (t, 3H, chain CH_3). MS (ESI) *m/z*: 509 $(M + H)^+$. Anal. Calcd. for $C_{32}H_{36}N_4O_2$: C, 75.55; H, 7.14; N, 11.02. Found: C, 75.57; H, 7.18; N, 11.05.

*6.1.6.6. (E)-N-(4-amino-2-methylquinolin-6-yl)-2-[4-(octylimino-methyl)phenoxymethyl]benzamide (**6f**).* Yield 79%, mp 74–75 °C ^{1}H NMR (DMSO-d_6) δ: 10.50 (s, 1H, NH), 8.36 (s, 1H, H-5), 8.19 (s, 1H, CH=N), 7.63 (m, 6 H, H-8, H-7, H-3′, H-6′, H-3″,5″), 7.52 (m, 2H, H-4′, H-5′), 7.01 (d, 2H, H-2″,6″), 6.44 (s, 1H, H-3), 6.38 (s, 2H, 4-NH_2),

5.38 (s, 2H, OCH_2), 3.48 (t, 2H, chain N–CH_2), 2.39 (s, 3H, 2-CH_3), 1.56 (m, 2H, chain CH_2), 1.24 (m, 10H, chain –$(CH_2)_5$–), 0.84 (t, 3H, chain CH_3). MS (ESI) *m/z*: 523 $(M + H)^+$. Anal. Calcd. for $C_{33}H_{38}N_4O_2$: C, 75.82; H, 7.33; N, 10.72. Found: C, 75.84; H, 7.35; N, 10.77.

*6.1.6.7. (E)-N-(4-amino-2-methylquinolin-6-yl)-2-[4-(nonylimino-methyl)phenoxymethyl]benzamide (**6g**).* Yield 80%, mp 95–98 °C ^{1}H NMR (DMSO-d_6) δ: 10.54 (s, 1H, NH), 8.38 (s, 1H, H-5), 8.20 (s, 1H, CH=N), 7.64 (m, 6 H, H-8, H-7, H-3′, H-6′, H-3″,5″), 7.52 (m, 2H, H-4′, H-5′), 7.01 (d, 2H, H-2″,6″), 6.44 (s, 1H, H-3), 6.41 (s, 2H, 4-NH_2), 5.39 (s, 2H, OCH_2), 3.48 (t, 2H, chain N–CH_2), 2.39 (s, 3H, 2-CH_3), 1.56 (m, 2H, chain CH_2), 1.25 (m, 12H, chain –$(CH_2)_6$–), 0.84 (t, 3H, chain CH_3). MS (ESI) *m/z*: 537 $(M + H)^+$. Anal. Calcd. for $C_{34}H_{40}N_4O_2$: C, 76.07; H, 7.52; N, 10.44. Found: C, 76.05; H, 7.48; N, 10.40.

*6.1.6.8. (E)-N-(4-amino-2-methylquinolin-6-yl)-2-[4-(decylimino-methyl)phenoxymethyl]benzamide (**6h**).* Yield 80%, mp 128–130 °C ^{1}H NMR (DMSO-d_6) δ: 10.53 (s, 1H, NH), 8.37 (s, 1H, H-5), 8.19 (s, 1H, CH=N), 7.63 (m, 6 H, H-8, H-7, H-3′, H-6′, H-3″,5″), 7.53 (m, 2H, H-4′, H-5′), 7.01 (d, 2H, H-2″,6″), 6.43 (s, 1H, H-3), 6.40 (s, 2H, 4-NH_2), 5.38 (s, 2H, OCH_2), 3.49 (t, 2H, chain N–CH_2), 2.39 (s, 3H, 2-CH_3), 1.55 (m, 2H, chain CH_2), 1.23 (m, 14H, chain –$(CH_2)_7$–), 0.85 (t, 3H, chain CH_3). MS (ESI) *m/z*: 551 $(M + H)^+$. Anal. Calcd. for $C_{35}H_{42}N_4O_2$: C, 76.32; H, 7.69; N, 10.18. Found: C, 76.33; H, 7.71; N, 10.15.

*6.1.6.9. N-(4-amino-2-methylquinolin-6-yl)-2-[4-(proylaminomethyl) phenoxymethyl]benzamide (**7a**).* Yield 92%, mp 118–120 °C ^{1}H NMR (DMSO-d_6) δ: 10.50 (s, 1H, NH), 8.38 (s, 1H, H-5), 7.63 (m, 4 H, H-8, H-7, H-3′, H-6′), 7.55 (m, 2H, H-4′, H-5′), 7.17 (d, 2H, H-3″,5″), 6.88 (d, 2H, H-2″,6″), 6.43 (s, 1H, H-3), 6.39 (s, 2H, 4-NH_2), 5.32 (s, 2H, OCH_2), 3.60 (s, 2H, 4″-NCH_2), 2.56 (t, 2H, chain N–CH_2), 2.40 (s, 3H, 2-CH_3,), 1.30 (m, 2H, chain CH_2), 1.22 (t, 3H, chain CH_3). MS (ESI) *m/z*: 455 $(M + H)^+$. Anal. Calcd. for $C_{28}H_{30}N_4O_2$: C, 73.97; H, 6.66; N, 12.33. Found: C, 73.93; H, 6.61; N, 12.31.

*6.1.6.10. N-(4-amino-2-methylquinolin-6-yl)-2-[4-(butylaminomethyl) phenoxymethyl]benzamide (**7b**).* Yield 92%, mp 106–108 °C ^{1}H NMR (DMSO-d_6) δ: 10.51 (s, 1H, NH), 8.38 (s, 1H, H-5), 7.65 (m, 4 H, H-8, H-7, H-3′, H-6′), 7.55 (m, 2H, H-4′, H-5′), 7.16 (d, 2H, H-3″,5″), 6.86 (d, 2H, H-2″,6″), 6.44 (s, 1H, H-3), 6.39 (s, 2H, 4-NH_2), 5.35 (s, 2H, OCH_2), 3.60 (s, 2H, 4″-NCH_2), 2.57 (t, 2H, chain N–CH_2), 2.40 (s, 3H, 2-CH_3,), 1.28 (m, 4H, chain $(CH_2)_2$–), 0.85 (bt, 3H, chain CH_3). MS (ESI) *m/z*: 469 $(M + H)^+$. Anal. Calcd. for $C_{29}H_{32}N_4O_2$: C, 74.32; H, 6.89; N, 11.96. Found: C, 74.36; H, 6.91; N, 11.95.

*6.1.6.11. N-(4-amino-2-methylquinolin-6-yl)-2-[4-(pentylaminomethyl) phenoxymethyl]benzamide (**7c**).* Yield 94%, mp 108–110 °C ^{1}H NMR (DMSO-d_6) δ: 10.50 (s, 1H, NH), 8.37 (s, 1H, H-5), 7.61 (m, 4 H, H-8, H-7, H-3′, H-6′), 7.50 (m, 2H, H-4′, H-5′), 7.16 (d, 2H, H-3″,5″), 6.89 (d, 2H, H-2″,6″), 6.43 (s, 1H, H-3), 6.40 (s, 2H, 4-NH_2), 5.30 (s, 2H, OCH_2), 3.58 (s, 2H, 4″-NCH_2), 2.58 (t, 2H, chain N–CH_2), 2.39 (s, 3H, 2-CH_3,), 1.21 (m, 6H, chain –$(CH_2)_3$–), 0.85 (bt, 3H, chain CH_3). MS (ESI) *m/z*: 483 $(M + H)^+$. Anal. Calcd. for $C_{30}H_{34}N_4O_2$: C, 74.65; H, 7.11; N, 11.61. Found: C, 74.67; H, 7.12; N, 11.60.

*6.1.6.12. N-(4-amino-2-methylquinolin-6-yl)-2-[4-(hexylaminomethyl) phenoxymethyl]benzamide (**7d**).* Yield 96%, mp 107–109 °C ^{1}H NMR (DMSO-d_6) δ: 10.51 (s, 1H, NH), 8.38 (s, 1H, H-5), 7.64 (m, 4 H, H-8, H-7, H-3′, H-6′), 7.52 (m, 2H, H-4′, H-5′), 7.21 (d, 2H, H-3″,5″), 6.91 (d, 2H, H-2″,6″), 6.44 (s, 1H, H-3), 6.41 (s, 2H, 4-NH_2), 5.31 (s, 2H, OCH_2), 3.67 (s, 2H, 4″-NCH_2), 2.58 (bs, 2H, chain N–CH_2), 2.40 (s, 3H, 2-CH_3,), 1.22 (m, 8H, chain –$(CH_2)_4$–), 0.84 (bt, 3H, chain CH_3). MS (ESI) *m/z*: 497 $(M + H)^+$. Anal. Calcd. for $C_{31}H_{36}N_4O_2$: C, 74.96; H, 7.31; N, 11.29. Found: C, 74.95; H, 7.33; N, 11.27.

*6.1.6.13. N-(4-amino-2-methylquinolin-6-yl)-2-[4-(heptylaminomethyl) phenoxymethyl]benzamide (**7e**).* Yield 94%, mp 64–66 °C ^{1}H NMR (DMSO-d_6) δ: 10.50 (s, 1H, NH), 8.38 (s, 1H, H-5), 7.64 (m, 4 H, H-8, H-7, H-3′, H-6′), 7.55 (m, 2H, H-4′, H-5′), 7.15 (d, 2H, H-3″,5″), 6.85 (d, 2H, H-2″,6″), 6.44 (s, 1H, H-3), 6.40 (s, 2H, 4-NH_2), 5.31 (s, 2H, OCH_2), 3.55 (s, 2H, 4″-NCH_2), 2.60 (bs, 2H, chain N–CH_2), 2.40 (s, 3H, 2-CH_3,), 1.23 (m, 10H, chain –$(CH_2)_5$–), 0.84 (bt, 3H, chain CH_3). MS (ESI) *m/z*: 511 $(M + H)^+$. Anal. Calcd. for $C_{32}H_{38}N_4O_2$: C, 75.25; H, 7.50; N, 10.98. Found: C, 75.27; H, 7.53; N, 10.93.

*6.1.6.14. N-(4-amino-2-methylquinolin-6-yl)-2-[4-(octylaminomethyl) phenoxymethyl]benzamide (**7f**).* Yield 92%, mp 63–65 °C ^{1}H NMR (DMSO-d_6) δ: 10.51 (s, 1H, NH), 8.38 (s, 1H, H-5), 7.65 (m, 4 H, H-8, H-7, H-3′, H-6′), 7.53 (m, 2H, H-4′, H-5′), 7.16 (d, 2H, H-3″,5″), 6.87 (d, 2H, H-2″,6″), 6.45 (s, 1H, H-3), 6.40 (s, 2H, 4-NH_2), 5.32 (s, 2H, OCH_2), 3.56 (s, 2H, 4″-NCH_2), 2.60 (bs, 2H, chain N–CH_2), 2.40 (s, 3H, 2-CH_3,), 1.23 (m, 12H, chain –$(CH_2)_6$–), 0.84 (bt, 3H, chain CH_3). MS (ESI) *m/z*: 525 $(M + H)^+$. Anal. Calcd. for $C_{33}H_{40}N_4O_2$: C, 75.53; H, 7.69; N, 10.68. Found: C, 75.56; H, 7.74; N, 10.66.

*6.1.6.15. N-(4-amino-2-methylquinolin-6-yl)-2-[4-(nonylaminomethyl) phenoxymethyl]benzamide (**7g**).* Yield 95%, mp 65–66 °C ^{1}H NMR (DMSO-d_6) δ: 10.52 (s, 1H, NH), 8.38 (s, 1H, H-5), 7.63 (m, 4H, H-8, H-7, H-3′, H-6′), 7.53 (m, 2H, H-4′, H-5′), 7.17 (d, 2H, H-3″,5″), 6.88 (d, 2H, H-2″,6″), 6.43 (s, 1H, H-3), 6.40 (s, 2H, 4-NH_2), 5.30 (s, 2H, OCH_2), 3.54 (s, 2H, 4″-NCH_2), 2.58 (bs, 2H, chain N–CH_2), 2.39 (s, 3H, 2-CH_3,), 1.22 (m, 14H, chain –$(CH_2)_7$–), 0.84 (bt, 3H, chain CH_3). MS (ESI) *m/z*: 539 $(M + H)^+$. Anal. Calcd. for $C_{34}H_{42}N_4O_2$: C, 75.79; H, 7.86; N, 10.40. Found: C, 75.82; H, 7.85; N, 10.42.

*6.1.6.16. N-(4-amino-2-methylquinolin-6-yl)-2-[4-(decylaminomethyl) phenoxymethyl]benzamide (**7h**).* Yield 97%, mp 65–67 °C ^{1}H NMR (DMSO-d_6) δ: 10.89 (s, 1H, NH), 8.73 (s, 1H, H-5), 8.63 (bs, 2H, 4-NH_2), 7.92 (bs, 2H, H-7, H-8), 7.58 (m, 4H, H-3′, H-4′, H-5′, H-6′), 7.37 (d, 2H, H-3″,5″), 6.98 (d, 2H, H-2″,6″), 6.60 (s, 1H, H-3), 5.36 (s, 2H, OCH_2), 3.99 (s, 2H, 4″-NCH_2), 2.79 (bs, 2H, chain N–CH_2), 2.59 (s, 3H, 2-CH_3,), 1.23 (m, 16H, chain –$(CH_2)_8$–), 0.85 (bt, 3H, chain CH_3). MS (ESI) *m/z*: 553 $(M + H)^+$. Anal. Calcd. for $C_{35}H_{44}N_4O_2$: C, 76.04; H, 8.03; N, 10.14. Found: C, 76.08; H, 8.02; N, 10.16.

*6.1.7. General procedure for the preparation of N-(4-amino-2-methyl-quinolin-6-yl)-2-[4-(ω-aminoalkylaminomethyl)phenoxymethyl] benzamide (**8**)*

Aldehyde **3** (0.3 g, 0.73 mmol), N-Fmoc-diamine.(used as hydrobromide for **8a**–**d** preparation and as free base for **8e**–**h** preparation) (0.73 mmol) and diisopropylethylamine (0.3 mL) were dissolved in methanol (10 mL), and the solution was kept under stirring for the weekend at 25 °C. The reaction was monitored by thin layer chromatography on alumina plates, eluting with an ethyl acetate – 5% methanol mixture. Reduction of the so obtained Schiff bases was subsequently made with sodium borohydride (0.3 g) at room temperature for 3 h. Finally, methanolic solution was concentrated and the residue was treated with water and extracted with ethyl acetate. Organic layer was evaporated affording the expected compounds *N*-(4-amino-2-methylquinolin-6-yl)-2-[4-(ω-Fmocaminoalkylaminomethyl)phenoxymethyl]benzamides.
Cleavage of Fmoc protecting group was achieved by a mixture of 20% piperidine/DMF (0.5 mL) at room temperature for 3 h. The resulting suspension was diluted with methanol, filtered and concentrated in vacuo. The residue was added with ethyl ether and shaken overnight. The ethereal phase containing the side-product fluorenylmethylpiperidine derived from the deprotection, was

removed away by decantation and the residue rinsed thoroughly or crystallized from ethyl ether.

*6.1.7.1. N-(4-amino-2-methylquinolin-6-yl)-2-[4-(3-aminopropylaminomethyl)phenoxymethyl]benzamide (**8a**).* Yield 30%, mp 110–112 °C ^{1}H NMR (DMSO-d_6) δ: 10.50 (s, 1H, NH), 8.37 (s, 1H, H-5), 7.64 (m, 4 H, H-8, H-7, H-3′, H-6′), 7.51 (m, 2H, H-4′, H-5′), 7.14 (d, 2H, H-3″,5″), 6.87 (d, 2H, H-2″,6″), 6.44 (s, 1H, H-3), 6.39 (s, 2H, 4-NH_2), 5.31 (s, 2H, OCH_2), 3.54 (s, 2H, 4″-NCH_2), 3.30 (b, 2H, chain NH_2), 2.49 (under DMSO, t, 2H, chain α-NCH_2), 2.39 (s, 3H, 2-CH_3,), 1.47 (m, 2H, chain α′−CH_2NH_2), 1.09 (m, 2H, chain β-CH_2). MS (ESI) *m/z*: 470 $(M + H)^+$. Anal. Calcd. for $C_{28}H_{31}N_5O_2$: C, 71.60; H, 6.66; N, 14.92. Found: C, 71.58; H, 6.67; N, 14.93.

*6.1.7.2. N-(4-amino-2-methylquinolin-6-yl)-2-[4-(4-aminobutylaminomethyl)phenoxymethyl]benzamide (**8b**).* Yield 25%, mp 103–105 °C ^{1}H NMR (DMSO-d_6) δ: 10.45 (s, 1H, NH), 8.35 (s, 1H, H-5), 7.63 (m, 4 H, H-8, H-7, H-3′, H-6′), 7.51 (m, 2H, H-4′, H-5′), 7.17 (d, 2H, H-3″,5″), 6.88 (d, 2H, H-2″,6″), 6.44 (s, 1H, H-3), 6.32 (s, 2H, 4-NH_2), 5.31 (s, 2H, OCH_2), 3.56 (s, 2H, 4″-NCH_2), 3.27 (b, 2H, chain NH_2), 2.42 (partially under DMSO, m, 4H, chain α,α′−NCH_2), 2.39 (s, 3H, 2-CH_3,), 1.38 (m, 4H, chain β, β′−CH_2). MS (ESI) *m/z*: 484 $(M + H)^+$. Anal. Calcd. For $C_{29}H_{33}N_5O_2$: C, 72.01; H, 6.88; N, 14.49. Found: C, 72.06; H, 6.86; N, 14.44.

*6.1.7.3. N-(4-amino-2-methylquinolin-6-yl)-2-[4-(5-aminopentylaminomethyl)phenoxymethyl]benzamide (**8c**).* Yield 25%, mp 102–104 °C ^{1}H NMR (DMSO-d_6) δ: 10.51 (s, 1H, NH), 8.38 (s, 1H, H-5), 7.64 (m, 4 H, H-8, H-7, H-3′, H-6′), 7.52 (m, 2H, H-4′, H-5′), 7.18 (d, 2H, H-3″,5″), 6.90 (d, 2H, H-2″,6″), 6.43 (s, 1H, H-3), 6.40 (s, 2H, 4-NH_2), 5.31 (s, 2H, OCH_2), 3.54 (s, 2H, 4″-NCH_2), 3.34 (b, 2H, chain NH_2), 2.42 (partially under DMSO, m, 4H, chain α,α′−NCH_2), 2.39 (s, 3H, 2-CH_3,), 1.32 (m, 4H, chain β, β′−CH_2), 1.09 (bm, 2H, chain central γ-CH_2). MS (ESI) *m/z*: 498 $(M + H)^+$. Anal. Calcd. for $C_{30}H_{35}N_5O_2$: C, 72.39; H, 7.09; N, 14.08. Found: C, 72.35; H, 7.04; N, 14.05.

*6.1.7.4. N-(4-amino-2-methylquinolin-6-yl)-2-[4-(6-aminohexylaminomethyl)phenoxymethyl]benzamide (**8d**).* Yield 32%, mp 90–92 °C ^{1}H NMR (DMSO-d_6) δ: 10.53 (s, 1H, NH), 8.40 (s, 1H, H-5), 7.64 (m, 4 H, H-8, H-7, H-3′, H-6′), 7.53 (m, 2H, H-4′, H-5′), 7.20 (d, 2H, H-3″,5″), 6.90 (d, 2H, H-2″,6″), 6.45 (s, 1H, H-3), 6.40 (s, 2H, 4-NH_2), 5.32 (s, 2H, OCH_2), 3.56 (s, 2H, 4″-NCH_2), 3.34 (b, 2H, chain NH_2), 2.42 (partially under DMSO, m, 4H, chain α,α′−NCH_2), 2.39 (s, 3H, 2-CH_3,), 1.38 (m, 4H, chain β, β′−CH_2), 1.24 (bm, 4H, chain central γ, γ′−CH_2). MS (ESI) *m/z*: 512 $(M + H)^+$. Anal. Calcd. for $C_{31}H_{37}N_5O_2$: C, 72.76; H, 7.29; N, 13.69. Found: C, 72.78; H, 7.26; N, 13.68.

*6.1.7.5. N-(4-amino-2-methylquinolin-6-yl)-2-[4-(7-aminoheptylaminomethyl)phenoxymethyl]benzamide (**8e**).* Yield 28%, mp 82–84 °C ^{1}H NMR (DMSO-d_6) δ: 10.54 (s, 1H, NH), 8.39 (s, 1H, H-5), 7.63 (m, 4 H, H-8, H-7, H-3′, H-6′), 7.54 (m, 2H, H-4′, H-5′), 7.19 (d, 2H, H-3″,5″), 6.89 (d, 2H, H-2″,6″), 6.43 (s, 1H, H-3), 6.40 (s, 2H, 4-NH_2), 5.34 (s, 2H, OCH_2), 3.58 (s, 2H, 4″-NCH_2), 3.34 (b, 2H, chain NH_2), 2.42 (partially under DMSO, m, 4H, chain α,α′−NCH_2), 2.39 (s, 3H, 2-CH_3,), 1.37 (m, 4H, chain β, β′−CH_2), 1.21 (bm, 6H, chain central $(CH_2)_3$). MS (ESI) *m/z*: 526 $(M + H)^+$. Anal. Calcd. for $C_{32}H_{39}N_5O_2$: C, 73.10; H, 7.48; N, 13.33. Found: C, 73.12; H, 7.46; N, 13.32.

*6.1.7.6. N-(4-amino-2-methylquinolin-6-yl)-2-[4-(8-aminooctylaminomethyl)phenoxymethyl]benzamide (**8f**).* Yield 26%, mp 95–97 °C ^{1}H NMR (DMSO-d_6) δ: 10.55 (s, 1H, NH), 8.38 (s, 1H, H-5), 7.64 (m, 4 H, H-8, H-7, H-3′, H-6′), 7.53 (m, 2H, H-4′, H-5′), 7.19 (d, 2H, H-3″,5″), 6.87 (d, 2H, H-2″,6″), 6.43 (s, 1H, H-3), 6.40 (s, 2H, 4-NH_2), 5.36 (s, 2H, OCH_2), 3.57 (s, 2H, 4″-NCH_2), 3.34 (b, 2H, chain NH_2), 2.42 (partially under DMSO, m, 4H, chain α,α′−NCH_2), 2.39 (s, 3H, 2-CH_3,), 1.39 (m, 4H, chain β, β′−CH_2), 1.22 (bm, 8H, chain central $(CH_2)_4$). MS (ESI) *m/z*: 540 $(M + H)^+$. Anal. Calcd. for $C_{33}H_{41}N_5O_2$: C, 73.43; H, 7.66; N, 12.98. Found: C, 73.40; H, 7.61; N, 12.93.

*6.1.7.7. N-(4-amino-2-methylquinolin-6-yl)-2-[4-(9-aminononylaminomethyl)phenoxymethyl]benzamide (**8g**).* Yield 25%, mp 88–90 °C ^{1}H NMR (DMSO-d_6) δ: 10.51 (s, 1H, NH), 8.38 (s, 1H, H-5), 7.64 (m, 4 H, H-8, H-7, H-3′, H-6′), 7.52 (m, 2H, H-4′, H-5′), 7.18 (d, 2H, H-3″,5″), 6.90 (d, 2H, H-2″,6″), 6.43 (s, 1H, H-3), 6.40 (s, 2H, 4-NH_2), 5.31 (s, 2H, OCH_2), 3.54 (s, 2H, 4″-NCH_2), 3.34 (b, 2H, chain NH_2), 2.42 (partially under DMSO, m, 4H, chain α,α′−NCH_2), 2.39 (s, 3H, 2-CH_3,), 1.32 (m, 12H, chain central $(CH_2)_6$), 1.09 (bm, 2H, chain central CH_2). MS (ESI) *m/z*: 554 $(M + H)^+$. Anal. Calcd. for $C_{34}H_{43}N_5O_2$: C, 73.73; H, 7.83; N, 12.65. Found: C, 73.75; H, 7.86; N, 12.63.

*6.1.7.8. N-(4-amino-2-methylquinolin-6-yl)-2-[4-(10-aminodecylaminomethyl)phenoxymethyl]benzamide (**8h**).* Yield 25%, mp 90–92 °C ^{1}H NMR (DMSO-d_6) δ: 10.54 (s, 1H, NH), 8.39 (s, 1H, H-5), 7.64 (m, 4 H, H-8, H-7, H-3′, H-6′), 7.51 (m, 2H, H-4′, H-5′), 7.20 (d, 2H, H-3″,5″), 6.91 (d, 2H, H-2″,6″), 6.42 (s, 1H, H-3), 6.39 (s, 2H, 4-NH_2), 5.32 (s, 2H, OCH_2), 3.55 (s, 2H, 4″-NCH_2), 3.32 (b, 2H, chain NH_2), 2.42 (partially under DMSO, m, 4H, chain α,α′−NCH_2), 2.40 (s, 3H, 2-CH_3,), 1.23 (m, 16H, chain central $(CH_2)_8$). MS (ESI) *m/z*: 568 $(M + H)^+$. Anal. Calcd. for $C_{35}H_{45}N_5O_2$: C, 74.03; H, 7.99; N, 12.34. Found: C, 74.01; H, 7.97; N, 12.33.

*6.1.8. Methyl 2-[4-(trifluoroacetylaminomethyl)phenoxy] methylbenzoate (**9**)*

A mixture of 2,2,2-trifluoro-N-[(4-hydroxyphenyl)methyl]acetamide (3.3 g, 15 mmol), methyl 2-(bromomethyl)benzoate (3.4 g, 15 mmol) and anhydrous K_2CO_3 (10.4 g, 75 mmol) in anhydrous DMF (50 mL) was kept under stirring at 30 °C for 4 h, then the mixture was filtered and evaporated in vacuo. The residue was then washed with water and dried under vacuum to obtain **9** (4.7 g, 85%); mp 98–100 °C; ^{1}H NMR (DMSO-d_6) δ: 9.92 (s, 1H, NH), 7.89 (d, 1H, H-6), 7.63 (sharp m, 2H, H-3,4), 7.48 (t, 1H, H-5), 7.18 (d, 2H, H-3′,5′), 6.97 (d, 2H, H-2′,6′), 5.39 (s, 2H, OCH_2), 4.30 (d, 2H, NCH_2), 3.80 (s, 3H, OCH_3). MS (ESI) *m/z*: 368 $(M + H)^+$. Anal. Calcd. for $C_{18}H_{16}F_3NO_4$: C, 58.84; H, 4.39; N, 3.81. Found: C, 58.77; H, 4.28; N, 3.85.

*6.1.9. 2-[4-(Aminomethyl)phenoxy]methylbenzoic acid (**10**)*

A solution of **9** (3.2 g, 8.7 mmol) in tetrahydrofuran (10 mL), methanol (10 mL) and 16% aqueous NaOH (6.5 mL) was heated at 90 °C under stirring for 3 h, then evaporated in vacuo to remove the organic solvents. The aqueous residue was then diluted with ice water, then 1M HCl was added dropwise until to pH 3–4, and the resulting white precipitate collected by filtration, washed with water and dried in vacuo at 50 °C to obtain **10** (1.4 g, 62%), mp 255–258 °C ^{1}H NMR (DMSO-d_6) δ: 8.13 (bs, 2H, NH_2), 7.92 (d, 1H, H-6), 7.58 (m, 2H, H-3 and H-4), 7.38 (m, 3H, H-5, H-3′,5′), 7.00 (d, 2H, H-2′,6′), 5.46 (s, 2H, OCH_2), 3.94 (d, 2H, NCH_2). MS (ESI) *m/z*: 258 $(M + H)^+$. Anal. Calcd. for $C_{15}H_{15}NO_3$: C, 70.01; H, 5.88; N, 5.45. Found: C, 70.16; H, 5.97; N, 5.53.

*6.1.10. 2-[4-(Trifluoroacetylaminomethyl)phenoxy]methylbenzoic acid (**11**)*

To a suspension of **10** (0.65 g, 2.5 mmol) in pyridine (2 mL) at 15 °C, N-ethyldiisopropylamine (0.41 mL, 2.5 mmol) was added, then a solution of trifluoroacetic anhydride (0.35 mL, 2.5 mmol) in CH_2Cl_2 (1 mL) was added dropwise under cooling and stirring. The stirring was continued overnight at room temperature, then ice water was added, followed to 1M HCl which was added dropwise to pH about 2. The resulting precipitate was collected by filtration, washed with water and dried in vacuo in an oven at 50 °C to give **11**

(0.36 g, 40%); mp 190–191 °C dec.; ^{1}H NMR (DMSO-d_6) δ: 9.94 (m, 1H, NH), 7.62 (m, 4H, H-3, H-4, H-5, H-6), 7.21 (d, 2H, H-3′,5′), 6.99 (d, 2H, H-2′,6′), 5.44 (s, 2H, OCH_2), 4.31 (d, 2H, NCH_2). MS (ESI) *m/z*: 354 $(M + H)^+$. Anal. Calcd. for $C_{17}H_{14}F_3NO_4$: C, 57.78; H, 4.00; N, 3.97. Found: C, 57.72; H, 3.89; N, 3.86.

6.1.11. *N-(4-amino-2-methylquinolin-6-yl)-2-[4-(trifluoroacetylaminomethyl)phenoxymethyl]benzamide (**12**)*

A suspension of **11** (0.60 g, 1.7 mmol) in oxalyl chloride (0.75 mL) and anhydrous toluene (20 mL) was stirred overnight at room temperature. The resulting clear solution was then evaporated in vacuo, and the raw acyl chloride so obtained was dissolved in CH_2Cl_2 (3 mL). The solution so obtained was slowly added to a solution 4,6-diamino-2-methylquinoline (0.30 g, 1.7 mmol) in pyridine (7.5 mL) at 0–5 °C under stirring. The mixture was kept overnight at 35 °C under stirring, then evaporated in vacuo. The solid residue suspended in water, collected by filtration, washed with water and then crystallized from ethyl acetate. Yield 0.45 g (52%); mp 164–165 °C. ^{1}H NMR (DMSO-d_6) δ: 10.91 (s, 1H, benzamide NH), 10.00 (t, 1H, acetamide NH), 8.74 (bs, 3H, H-5 and 4-NH_2), 7.99 (dd, 1H, H-7), 7.65 (m, 5H, H-8, H-3′, H-4′, H-5′, H-6′), 7.16 (d, 2H, H-3″,5″), 6.93 (d, 2H, H-2″,6″), 6.64 (s, 1H, H-3), 5.34 (s, 2H, OCH_2), 4.28 (d, 2H, 4″-CH_2), 2.61 (s, 3H, 2-CH_3). MS (ESI) *m/z*: 509 $(M + H)^+$. Anal. Calcd. for $C_{27}H_{23}F_3N_4O_3$: C, 63.76; H, 4.56; N, 11.02. Found: C, 63.65; H, 4.72; N, 10.98.

6.1.12. *N-(4-amino-2-methylquinolin-6-yl)-2-[4-(aminomethyl) phenoxymethyl]benzamide (**13**)*

A solution of **12** (0.20 g, 0.4 mmol) in saturated methanolic ammonia (4 mL) was kept overnight at room temperature; the solvent was then removed in vacuo and the residue suspended in diethyl ether, collected by filtration, washed with diethyl ether and then crystallized from ethyl acetate with some drop of methanol. Yield 0.15 g (92%), mp 213–215 °C ^{1}H NMR (DMSO-d_6) δ: 10.53 (s, 1H, NH), 8.39 (s, 1H, H-5), 7.65 (sharp m, 5H, H-8, H-7, H-4′, H-5′, H-6′), 7.53 (d, 1H, H-3′), 7.20 (d, 2H, H-3″,5″), 6.90 (d, 2H, H-2″,6″), 6.45 (s, 1H, H-3), 6.42 (bs, 2H, 4-NH_2), 5.33 (s, 2H, OCH_2), 3.62 (s, 2H, 4″-CH_2), 2.40 (s, 3H, 2-CH_3). MS (ESI) *m/z*: 413 $(M + H)^+$. Anal. Calcd. for $C_{25}H_{24}N_4O_2$: C, 72.78; H, 5.87; N, 13.59. Found: C, 72.84; H, 6.04; N, 13.50.

6.2. Molecular Modeling

Minimum energy conformations of the compounds were generated by Molecular Modeling Software Chem-X (Chemical Design, Ltd.) and used to measure intramolecular distances. At the lowest energy the molecules showed a stretched molecular shape.

6.3. Pharmacology

6.3.1. *General procedure for NOP Receptor binding assay in membrane preparations*

Enriched plasma membranes from transfected cells were prepared by differential centrifugations and stored at −80 °C (protein concentration 2–4 mg/mL) until used. The binding of ^{125}I-Tyr$_{14}$-nociceptin (Perkin Elmer Life Sciences) was measured in 1 mL reaction mixture containing 50 mM Hepes-Tris pH 7.4, 10 mM $MgSO_4$, 10 μM leupeptin, 10 μM bestatin, 0.1 mg/mL bacitracin, 0.1% (w/v) bovine serum albumin (BSA), and 3 μg of membrane proteins from HEK-293 cells transfected with the NOP receptor as previously described [16]. The concentration of radiotracer was maintained constant at 50,000–100,000 cpm in the presence of increasing concentrations of compounds to be tested. Reactions lasted 90 min at room temperature and were terminated by rapid filtration onto GF/B glass fiber filtering microplates pre-treated with 0.1% ethylene imine polymer (PESI) (Filtermate 196; Packard Instruments, Meriden, CT). Filters were washed three times with 1 mL of ice-cold 50 mM Tris–HCl pH 7.4 and allowed to dry for 30 min at 37 °C. The plates were counted in a Top Count (Packard Instruments) after the addition (50 μl) of Microscint 20 (Packard) to each well.

IC_{50} values were obtained by fitting the competition curves according to a 4-parameter logistic model (ALLFIT program) [20].

6.3.2. *General procedure for δ, κ, μ receptors binding assay*

The binding affinities for human δ, κ and μ receptors were determined with [^{3}H]-naltrindole for δ receptor and with [^{3}H]-diprenorphine for κ and μ receptors [17,18].

A suspension of membranes (5–15 μg) of HEK-293 cells expressing δ receptors [18] and κ receptors (Perkin Elmer Life Sciences) and of CHO cells expressing μ receptors (Perkin Elmer Life Sciences) was incubated with the radioligand (30,000–50,000 cpm) at room temperature for 2 h in a total volume of 1 mL containing 50 mM Hepes-Tris pH 7.4, 100 mM NaCl, 0.1 mg/mL bacitracin, 0.1% (w/v) BSA and increasing concentrations of compounds to be tested. Samples were incubated for 90 min at room temperature, filtered onto GF/B glass fiber filtering microplates (Filtermate 196; Packard Instruments, Meriden, CT) and washed three times with 1 mL of ice-cold 50 mM Tris–HCl pH 7.4 prior to scintillation counting on a Packard Top Count.

IC_{50} values were obtained by fitting the competition curves according to a 4-parameter logistic model (ALLFIT program) [20].

6.3.3. *GTPγS binding*

The ^{35}S GTPγS binding was determined in a 1 mL reaction mixture containing 50 mM Hepes-Tris pH 7.4, 1 mM Dithiothreitol (DTT), 100 mM NaCl, 10 mM $MgSO_4$, 0.1 nM ^{35}S GTPγS (Perkin Elmer Life Sciences), 300 nM GDP (or concentrations varying between 0.1 nM and 100 μM) and 1–2 μg of membrane proteins, with or without compounds to be tested [16].

Acknowledgments

We wish to thank Dr. A. Alvino (Department of Chemistry, University “La Sapienza” – Roma) for mass spectra and Mr. A. Puccio for technical assistance.

References

[1] C. Mollereau, M. Parmentier, P. Mailleux, J.L. Butour, C. Moisand, P. Chalon, D. Caput, G. Vassart, J.C. Meunier, ORL1, a novel member of the opioid receptor family: cloning, functional expression and localization, FEBS Lett. 341 (1994) 33–38.
[2] D.G. Lambert, The nociceptin/orphanin FQ receptor: a target with broad therapeutic potential, Nat. Rev. Drug Discov. 7 (2008) 694–710.
[3] H. Shinkai, T. Ito, T. Iida, Y. Kitao, H. Yamada, I. Uchida, 4-Aminoquinolines: novel nociceptin antagonists with analgesic activity, J. Med. Chem. 43 (2000) 4667–4677.
[4] H. Yamada, H. Nakamoto, Y. Suzuki, T. Ito, K. Aisaka, Pharmacological profiles of a novel opioid-receptor-like 1 (ORL1) receptor antagonist, JTC-801, Br. J. Pharmacol. 135 (2002) 323–332.
[5] I. Sestili, A. Borioni, C. Mustazza, A. Rodomonte, L. Turchetto, M. Sbraccia, D. Riitano, M.R.Del Giudice, A new synthetic approach of *N*-(4-amino-2-methylquinolin-6-yl)-2-(4-ethylphenoxymethyl)benzamide (JTC-801) and its analogues and their pharmacological evaluation as nociceptin receptor (NOP) antagonists, Eur. J. Med. Chem. 39 (2004) 1047–1057.
[6] J.M. Contreras, J.J. Bourguignon, Identical and non-identical twin drugs. in: C.G. Wermuth (Ed.), The Practice of Medicinal Chemistry. Academic Press, London, 2003, pp. 251–273.
[7] N. Dietis, R. Guerrini, G. Calò, S. Salvadori, D.J. Rowbotham, D.G. Lambert, Simultaneous targeting of multiple opioid receptors: a strategy to improve side-effect profile, Br. J. Anaesth. 103 (2009) 38–49.
[8] P. Nair, T. Yamamoto, V. Kulkarni, S. Moye, E. Navratilova, P. Davis, T. Largent, S. Ma, H.I. Yamamura, T. Vanderah, J. Lai, F. Porreca, V.J. Hruby, Novel bifunctional peptides as opioid agonists and NK-1 antagonists, Adv. Exp. Med. Biol. 611 (2009) 537–538.

[9] R. Vardanyan, G. Vijay, G.S. Nichol, L. Liu, I. Kumarasinghe, P. Davis, T. Vanderah, F. Porreca, J. Lai, V.J. Hruby, Synthesis and investigations of double-pharmacophore ligands for treatment of chronic and neuropathic pain, Bioorg. Med. Chem. 17 (2009) 5044–5053.
[10] B.A. Jordan, S. Cvejic, L.A. Devi, Opioid and their complicated receptor complexes, Neuropsychopharmacology 23 (2000) S5–S18.
[11] P.S. Portoghese, From models to molecules: opioid receptor dimers, bivalent ligands, and selective opioid receptor probes, J. Med. Chem. 44 (2001) 2259–2269.
[12] S. Ballet, M. Pietsch, A.D. Abell, Multiple ligands in opioid research, Protein Pept. Lett. 15 (2008) 668–682.
[13] L.B.L. Hansen, B.D. Larsen, C. Thorkildsen, C.B. Knudsen (Clark & Elbing LLP), Nociceptin Analogues and Uses Thereof. U.S. 0287478 A1, 2008.
[14] F. Jenck, J. Wichmann, F.M. Dautzenberg, J.L. Moureau, A.M. Ouagazzal, J.R. Martin, K. Lundstrom, A.M. Cesura, S.M. Poli, S. Röver, S. Kolczewski, G. Adam, G.A. Kilpatrick, A synthetic agonist at the orphanin FQ/ nociceptin receptor ORL1: anxiolytic profile in the rat, Proc. Natl. Acad. Sci. U S A. 97 (2000) 4938–4943.
[15] J.G. Allen, K. Briner, M.P. Cohen, C. Galka, S.L. Hellman, M.A. Martinez-Grau, M.R. Reinhard, M.J. Rodriguez, R.R. Rothhaar, M.W. Tidwell, F. Victor, A.C. Williams, D. Zhang, S.A. Boyd, R.G. Conway, A.S. Deo, W.M. Lee, C.S. Siedem, A. Singh (Eli Lilly and Co.), 6-Substituted-2,3,4,5-tetrahydro-1H-benzo[*d*]azepines as 5–HT2C receptor agonists. WO 2005/082859 A1, 2005.
[16] P. Molinari, C. Ambrosio, D. Riitano, M. Sbraccia, M.C. Grò, T. Costa, The receptor of one covalent complex interacts with the α-subunit of another, J. Biol. Chem. 278 (2003) 15778–15788.
[17] F. Simonin, C. Gavériaux-Ruff, K. Befort, H. Matthes, B. Lannes, G. Micheletti, M.G. Mattéi, G. Charron, B. Bloch, B. Kieffer, Kappa-opioid receptor in humans: cDNA and genomic cloning, chromosomal assignment, functional expression, pharmacology, and expression pattern in the central nervous system, Proc. Natl. Acad. Sci. U S A 92 (1995) 7006–7010.
[18] E. Malatynska, Y. Wang, R.J. Knapp, G. Santoro, X. Li, S. Waite, W.R. Roeske, H.I. Yamamura, Human δ opioid receptor: a stable cell line for functional studies of opioids, Neuroreport 6 (1995) 613–616.
[19] B.M. Bröer, M. Gurrath, H.D. Höltje, Molecular modelling studies on the ORL1-receptor and ORL1-agonists, J. Comput. Aided Mol. Des. 17 (2003) 739–754.
[20] A. DeLean, P.J. Munson, D. Rodbard, Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay, and physiological dose-response curves, Am. J. Physiol. 235 (1978) E97–E102P.