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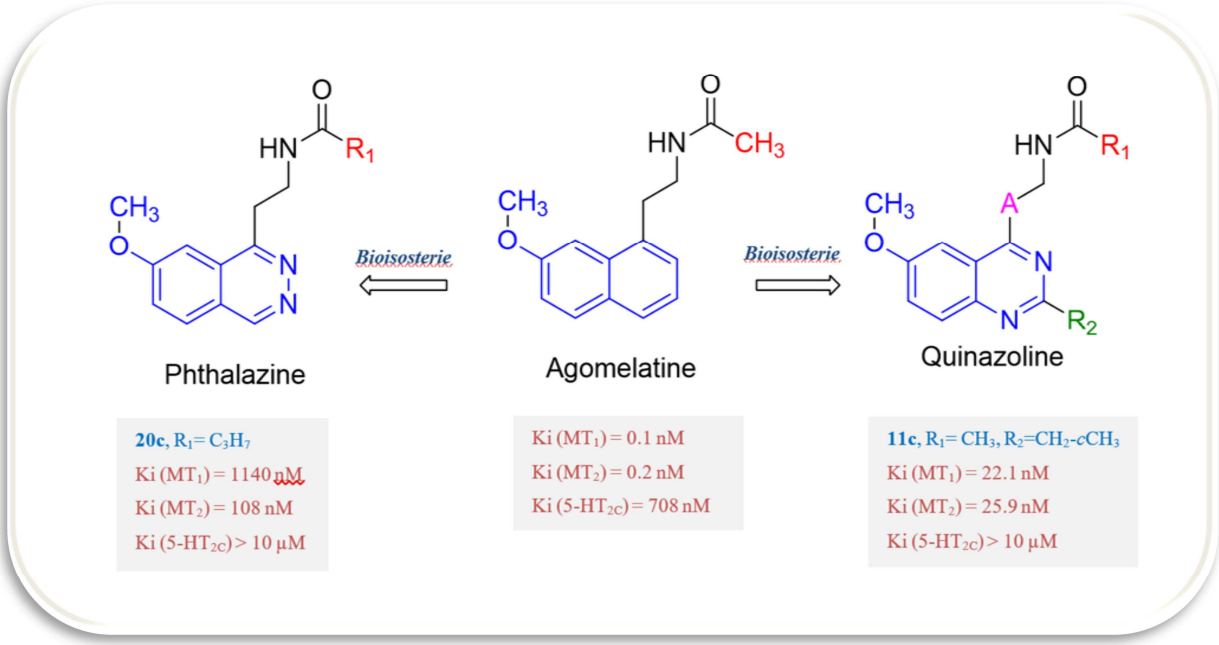
Graphical Abstract



Quinazoline and Phthalazine Derivatives as Novel Melatonin Receptor Ligands Analogues of Agomelatine

Raphaël Bolteau,[a⊥] Florian Descamps,[a⊥] Mohamed Ettaoussi,[a*] Daniel H Caignard,[b,c] Philippe Delagrange,[b] Patricia Melnyk,[a] and Saïd Yous[a*]

[a]*Univ. Lille, Inserm, CHU Lille, UMR-S1172 - JPArc - Centre de Recherche Jean-Pierre AUBERT Neurosciences et Cancer, F-59000 Lille, France*

[b]*PEX Biotechnologie Chimie & Biologie, Institut de Recherches Servier, 78290 Croissy sur Seine, France*

[c]*Institut de Recherches Internationales Servier, 92150 Suresnes, France*

[⊥]*These authors contributed equally*

Corresponding Authors:

**Corresponding author. [a]University of Lille, Inserm, CHU Lille, UMR-S1172 - JPArc - Centre de Recherche Jean-Pierre AUBERT Neurosciences et Cancer, F-59000 Lille, France. Emails: m.ettaoussi@yahoo.fr; said.yous@univ-lille2.fr*

Abstract

For further development of successors of Agomelatine through modulation of its pharmacokinetic properties, we report herein the design, synthesis and pharmacological results of a new family of melatonin receptor ligands. Issued from the introduction of quinazoline and phthalazine scaffolds carrying an ethyl amide lateral chain and a methoxy group as bioisosteric ligands analogs of previously developed Agomelatine. The biological activity of the prepared analogs was compared with that of Agomelatine. Quinazoline and phthalazine rings proved to be a versatile scaffold for easy feasible MT_1 and MT_2 ligands. Potent agonists with sub-micromolar binding affinity were obtained. However, the presence of two nitrogen atoms resulted in compounds with lower affinity for both MT_1 and MT_2, in comparison with the parent compound, balanced by the exhibition of good pharmacokinetic properties.

Key words

Agomelatine, agonist, melatonin receptor, MT_1, MT_2, quinazoline, phthalazine

Abbreviations used

AGM, Agomelatine; MLT, Melatonin; MT_1, melatonin receptor subtype 1; MT_2, melatonin receptor subtype 2

1. Introduction

Melatonin (MLT) is a neurohormone mainly synthesized by the pineal gland following a circadian rhythm [1]. This rhythmicity is important for melatonin physiological functions [2]. Once synthesized, melatonin is then released into the bloodstream and travels to different organs and tissues of the body [1] to obtain desirable physiological responses mainly on the central nervous system (CNS) [3]. MLT regulates a variety of physiological functions through the activation of two membrane receptors MT_1 and MT_2 [4]. Both, MT_1 and MT_2 receptor subtypes are classified as class A G-protein coupled receptors (GPCRs). Since its discovery, several studies have demonstrated the involvement of MLT and its receptors in the regulation of different physiological processes such as sleep and wakefulness [5], core body temperature and blood pressure [6]. Some studies have demonstrated possible role of MT_1 and/or MT_2 receptors in several disorders including insomnia and mood disorders, depression, anxiety, Alzheimer, Parkinson's diseases [7]. Melatonin was also demonstrated to possess antioxidant and anti-inflammatory effects [8].

Consequently, the melatonergic system represents an interesting target for the development of new therapeutic agents. Accordingly, research for melatonin receptor ligands has led to the design and synthesis of several ligands. Many of these ligands have been or are in their way to be used as therapeutic agents. Besides, Ramelteon a high affinity nonselective MT_1/MT_2 receptor agonist was developed and commercialized for the treatment of insomnia and sleep disorders [9]. More interestingly, the discovery of Agomelatine (AGM) was ingenious because of its unique pharmacological profile. AGM is a high-affinity nonselective MT_1/MT_2 melatonin receptor agonist and 5-HT_{2C} serotonin receptor selective antagonist. It is marketed as an antidepressant with a high efficacy on sleep, circadian rhythm disorder, and depression [10]. However, up to recently the absence of experimental 3D models of MT_1 and MT_2 hindered the structure-based drug design. Indeed, melatonin receptor models were

constructed and refined using 3D structures of different templates including serotonin receptors such as the human 5-HT_{1B} and 5-HT_{2B} [11]. Fortunately, very recently a group of researchers described high-resolution XFEL (X-ray free electron laser) structures of the human MT_1 receptor in complex with ramelteon, 2-phenylmelatonin, 2-iodomelatonin, and AGM; and the human MT_2 receptor in complex with 2-phenylmelatonin (2-PMT) and ramelteon [12]. Progress in understanding the role of melatonin receptors will be boosted by these structural findings of the MT_1 and MT_2 receptors subtypes. Moreover, the determination of the human MT_2 melatonin receptor subtype selectivity reported in this work will lead certainly to the design and synthesis of highly selective ligands and hence the comprehension of this system.

Following our contribution to the understanding of the melatonin system, we reported the design and synthesis of analogues of agomelatine issued from the replacement of the naphthalene scaffold by different heterocycles (Figure 1) [13]. Herein we report the synthesis and the pharmacological evaluation of two aza-cycles bioisosteric analogues of AGM. Quinazoline and phthalazine are two heterocycles that attracted much interest among the scientific community and have been under deep investigation due to their different biological properties. Besides, phthalazine was extensively used in the preparation of a variety of bioactive molecules with different pharmacological properties including antimicrobial, antibacterial, cardio tonic, anticancer, antifungal, vasodilatation activity, antimalarial, anticonvulsant, and anti-inflammatory activities [14]. On the other hand, quinazoline is a pharmacologically promising heterocycle due to its different biomedical properties. This heterocycle is present in different powerful antibacterial, antiviral, antifungal, anti-inflammatory, antimalarial, antitubercular and anti-HIV agents [14]. It is also present in some inhibitors of the epidermal growth factor (EGF) receptors of tyrosine kinase. Quinazolines were also employed as ligands for benzodiazepine and GABA-receptors in the CNS system or

as DNA binders, and as anticancer with remarkable activity [15]. Our main objective was to investigate the effect of the pharmacokinetic properties modulation via the introduction of two nitrogen atoms into the naphthalene cycle.

2. Results and discussion

2.1. Chemistry

Synthesis of amides **7a-d** is depicted in Scheme 1. First, key intermediate **3** was prepared following two synthetic routes. Using 5-methoxytryptamine, the primary amine was protected with a phthalimide group to give intermediate **1**. Oxidative cleavage of the indole heterocycle into compound **2** was achieved using $NaIO_4$ in methanol [16]. Quinazoline **3** was then obtained by cyclization of **2** in the presence of ammonium formate in formamide. The second method employed the commercially available 6-methoxyquinazolin-2-one as starting material. Chlorination of 6-methoxyquinazolin-2-one by $POCl_3$ gave 4-chloroquinazoline **4**. Heck cross-coupling reaction of **4** with *N*-vinylphthalimide in the presence of palladium acetate and tri(*o*-tolyl)phosphine [17] led to the unsaturated compound **5**. Hydrogenation of this compound gave the desired quinazoline **3**. Deprotection of the phthalimide group via hydrazinolysis in ethanol furnished the primary amine **6**. Finally, target amides **7a-d** were synthesized from **6** by reaction with the appropriate acid chloride [18].

<Insert Scheme 1 here>

Synthesis of 2,4,6-trisubstituted quinazolines **11a-c** is described in Scheme 2. First, kinurenamine **8** was obtained by oxidative cleavage of melatonin under the same conditions previously described for compound **2** [16]. Compound **8** was then submitted to a selective acidic hydrolysis of its formamide function leading to primary amine **9**. *N*-acylation of compound **9** by treatment with different acyl chlorides leads to amides **10a-c**. Substituted quinazolines **11a-c** were obtained by cyclization of compounds **10a-c** under the same

conditions as previously described for compound **3**. Moreover, this method also allowed access to the acetamide **7a** previously described in Scheme 1 directly from kinurenamine **8**. Amino-quinazoline **12** and piperazino-quinazolines **13-14** were prepared in one step (Scheme 3). Reaction of commercially available *N*-acetylethylene-diamine, 1-(cyclopropanoyl)-piperazine and *N*-ethylpiperazine-1-carboxamide with 4-chloro-6-methoxyquinazoline **4** at room temperature in the presence of triethylamine yielded the desired quinazolines **12-14** in good yields.

<Insert Scheme 2 here>

<Insert Scheme 3 here>

The phthalazine derivatives were prepared following the method described in Scheme 4. Therefore, phthalazines **20a-d** were prepared in six steps starting from commercially available 2-formyl-5-methoxybenzoic acid. First, condensation of this later compound with hydrazine in EtOH led to 7-methoxyphthalazin-(2*H*)-one (**15)** [19]. Chlorination of **15** with phosphorous oxychloride followed by treatment with ethyl cyanoacetate in the presence of sodium hydride (NaH) produced the cyanoester **17**. Refluxing of compound **17** in an acidic medium (5M HCl) led, after decarboxylation, to cyanomethyl **18**. Selective catalytic hydrogenation of the cyano group of **18**, with Raney-nickel, provided the aminoethyl phthalazine **19** which was then transformed into the desired amides **20a-d** according to a variant of the Schotten-Baumann procedure, by reaction with the appropriate acid chloride in the presence of potassium carbonate in a biphasic medium (ethyl acetate/water).

<Insert Scheme 4 here>

2.2. Pharmacology

<Insert Table 1 here>

First, all synthesized compounds were submitted to binding affinity experiments to the serotonergic 5-HT_{2C} receptor subtype using Chinese Hamster Ovarian (CHO) cell lines stably

expressing the human 5-HT_{2C} receptors and revealed the absence of any binding affinity or activity towards this receptor subtype. Second, binding assays for melatonin MT_1 and MT_2 receptor subtypes consisted of addition of membrane preparations from transfected CHO cells stably expressing the human melatonin MT_1 or MT_2 diluted in binding buffer (50 mM Tris–HCl buffer, pH 7.4, containing 5 mM $MgCl_2$) to 2-[^{125}I]iodomelatonin (20 pM for MT_1 and MT_2 receptors expressed in CHO cells) and the tested compound. Non-specific binding was defined in the presence of 1 µM melatonin, and data from the dose-response curves were analyzed using the program PRISM (Graph Pad Software Inc., San Diego, CA, USA) to yield IC_{50}. Results are expressed as pKi (pKi = -Log10 (Ki)) with Ki = IC_{50}/1 + ([L]/KD), where [L] is the concentration of radioligand used in the assay and KD, the dissociation constant of the radioligand characterizing the membrane preparation [21]. Evaluation of the prepared quinazoline and phthalazine derivatives for their affinities for melatonin receptors MT_1 and MT_2 receptors subtypes using transfected CHO cells stably expressing the human melatonin MT_1 or MT_2, revealed that strict bioisosteric replacement of the naphthalene moiety of AGM with quinazoline or phthalazine derivatives decreased the affinity for both melatonergic receptors MT_1 and MT_2 and showed weak MT_2-selectivity (**7a**, Table 1; **20**, Table 2). To further investigate the effect of this pharmacomodulation on the melatonergic affinity, we then analysed the effect of the replacement of the acetamide function of **7a** by an ethylamide (**7b**) and a propyl amide (**7c**). The obtained results showed a conservation of the MT_1 affinity and an improvement of MT_2 binding affinity in comparison with the acetamide **7a**. However, the introduction of a cyclopropylamide (**7c**) decreased the binding affinities at both MT_1 and the MT_2 (Table 1).

Further modulations of **7a** were performed specially the introduction of aromatic substituents at position C-2 of the quinazoline ring the equivalent position to C-3 of

agomelatine, site to hydroxylation during its metabolism. First, the introduction of a phenyl (**11a**) or a *m*-methoxyphenyl (**11b**) has led to an increase of the MT_1 and a decrease of MT_2 binding affinities and the improvement of the MT_2-selectivity (57 and 11-folds respectively) in comparison with the parent compound **7a** (Table 1). In contrary, the introduction of a methyl cyclopropyl group (**11c**) showed the most interesting results of this series, an increase in both MT_1 and MT_2 binding affinities in comparison with the parent **7a**, and in accordance with the previously observed effects on the naphthalene derivatives [20]. The ethyl amide side chain was also replaced by an aminoethyl amide chain (**12**) or piperazine derivatives (**13-14**). These two modulations have led to a loss in the melatonergic affinity as shown in Table 1. This loss may result from the addition of a H-bond donor/acceptor (compound **12**) and a molecular restriction (compounds **13-14**) which hinder the free rotation of the lateral chain and hence disturb its binding to the receptor.

<Insert Table 2 here>

The observed decrease of the melatonergic affinity by the replacement of the naphthalene scaffold with quinazoline was confirmed with the second modulation. In fact, the modification of the two nitrogen atoms pattern via the introduction of a phthalazine ring proved to be unfavourable for the melatonergic affinity. Accordingly, compound **20a**, the strict bioisostere of the AGM, showed a significant decrease in affinity for both MT_1 and MT_2 receptors in comparison with agomelatine and quinazoline **7a**. Further modulations of the acetamide group had no positive effect on the affinity (Table 2). The replacement of the acetamide with an ethyl or a propyl group (compounds **20b-d**) showed a weak improvement of both melatonin MT_1 and MT_2 affinities in comparison with the acetamide **20a**, and the conservation of a weak MT_2 selectivity over MT_1. Finally, the replacement of the acetamide over a cyclopropyl amide **20d** has led to the drop in both MT_1 and MT_2 affinities (Table 2).

<Insert Table 3 here>

In addition, the intrinsic activities of the synthesized compounds were determined. The obtained results revealed that most compounds behaved as MT_1/MT_2 full agonists except **11b** (Table 3). Compound **11b** bearing a methoxyphenyl in the C2-position exhibited an MT_1 full agonist and MT_2 partial agonist activity. Such a pharmacological profile is very sought in pharmacology as a tool to assess the role of each receptor subtype. Moreover, to investigate the pharmacokinetics of the prepared compounds, the calculation of the logP (ClogP) was realized using Data Warrior software. The obtained results showed that due to the presence of more nitrogen atoms in the skeleton, quinazoline and phthalazine derivatives are more hydrophilic than agomelatine. This high hydrophilicity may grant good solubility and absorption to these compounds and hence improve their pharmacokinetic properties (Table 4). On another important aspect, drug absorption and permeability test (Caco2 test) of the most interesting prepared compounds is shown in Table 5. Obtained results showed a 100% absorption for the tested compounds except for the phthalazine **20a** which exhibit only 2% absorption hence confirming the ClogP results above (Table 4). Bioavailability results (Table 5) showed in general a good bioavailability in human and rodents for the tested compounds except compound **11a** which exhibits a very poor bioavailability of only 2 to 7% due to the presence of the sterically hindered phenyl in the C-2 position of the quinazoline ring.

<Insert Table 4 here>

<Insert Table 5 here>

3. Conclusions

Herein we investigated the effect of bioisosteric modulation of agomelatine through replacement of the naphthalene moiety by quinazoline and phthalazine nucleus. Quinazoline derivatives (**7a**-**14**) showed a decrease in the affinity for both MT_1 and MT_2 and the appearance of an MT_2-selectivty. The modulation of the C2-position of quinazoline led to

some interesting compounds with pronounced MT_2-selectivty for compounds **11a-11b**. Conversely to quinazoline, the introduction of phthalazine decreased dramatically the melatonergic affinity for both receptors. In fact, the introduction of two nitrogen atoms in the naphthalene led to the drop of the melatonergic affinity. The disposition of the two nitrogen seems to play a role in this decrease in the affinity. More interestingly, in general most tested compounds showed good absorption and bioavailability. Finally, with the recent exciting news of the description of both binding sites of melatonin MT_1 and MT_2 receptors, our group is planning to use this new data to construct new 3D models and probe the binding modes of these melatonin ligands. This model is primordial to explain why the introduction of nitrogen atoms to the aromatic cycle bearing the acetamide lateral chain led to the drop of the melatonergic binding affinity.

4. Experimental Section

4.1. Chemistry

Chemicals and solvents were obtained from commercial sources and used without further purification unless otherwise noted. Reactions were monitored by TLC performed on Macherey–Nagel Alugram® Sil 60/UV254 sheets (thickness 0.2 mm). Purification of products was carried out by recrystallization or column chromatography. Column chromatography was carried out using Macherey–Nagel silica gel (230–400 mesh). Melting points were determined on a Büchi SMP-20 capillary apparatus and are uncorrected. FT-IR spectra were recorded on a Thermo Nicolet Avatar 320 FT-IR spectrometer. NMR spectra were recorded on a Bruker DRX 300 spectrometer (operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C). Chemical shifts are expressed in ppm relative to either tetramethylsilane (TMS). Chemical shifts are reported as position (δ in ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, dd = double doublet, br = broad and m = multiplet), coupling

constant (*J* in Hz), relative integral and assignment. The purity of final compounds was determined by high pressure liquid chromatography (HPLC) using two columns: C18 Interchrom UPTISPHERE. The HPLC analysis was carried out on a Shimadzu LC-2010AHT system equipped with a UV detector set at 254 and 215 nm. The compounds were dissolved in 100 mL of buffer B and 900 mL of buffer A. The eluent system used was: buffer A (H_2O/TFA, 100:0.1) and buffer B (ACN/H_2O/TFA, 80:20:0.1). Retention times (tr) were obtained at a flow rate of 0.2 mL/min for 37 min using a gradient form 100% of buffer A over 1 min, to 100% buffer B over the next 30 min, to 100% of buffer A over 1 min and 100% of buffer A over 1 min. All tested compounds showed a purity of > 95%. The melting point analyses were performed on Barnstead Electrothermal Melting Point Series IA9200 and were not corrected.

4.1.1. 2-(2-(5-Methoxy-1H-indol-3-yl)ethyl)isoindoline-1,3-dione **(1)**. To a solution of 5-methoxytryptamine (800 mg, 4.2 mmol) in toluene (30 mL) was added phthalic anhydride (686 mg, 4.62 mmol) and Et_3N (0.64 mL, 4.62 mmol). The reaction mixture was heated at reflux for 24 h with continuous removal of water with a Dean−Stark trap. The mixture was cooled to room temperature and concentrated under *vacuum*. The residue was putted into 1M NaOH (20 mL) and extracted with DCM (3 x 100 mL). The combined organic layers were washed with 1M HCl and water. The organic phase was dried with $MgSO_4$, filtered, and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography (PE/AcOEt); 10:0 to 7:3 (*v/v*)) to afford **1** as a beige powder (87% yield); mp 165 °C; ^{1}H NMR (300 MHz, CD_2Cl_2): δ 8.08 (br s, 1H), 7.86 – 7.80 (m, 2H), 7.88 – 7.69 (m, 2H), 7.26 (d, *J* = 8.8 Hz, 1H), 7.15 (d, *J* = 2.5 Hz, 1H), 7.09 (d, *J* = 2.1 Hz, 1H), 6.82 (dd, *J* = 8.8, 2.5 Hz, 1H), 3.99 (m, 2H), 3.84 (s, 3H), 3.13 (m, 2H); ^{13}C NMR (75 MHz, CD_2Cl_2): δ 168.2, 154.0, 133.9, 132.2, 131.3, 127.8, 122.9, 112.2, 111.8, 100.3, 55.6, 38.4, 24.3

4.1.2. N-(2-(3-(1,3-Dioxoisoindolin-2-yl)propanoyl)-4-methoxyphenyl)formamide **(2)**. To solution of **1** (437 mg, 1.36 mmol) in methanol (26 mL) was added dropwise a solution of $NaIO_4$ (1.17 g, 5.46 mmol) in water (30 mL) at 0 °C. The mixture was stirred at 80 °C for 12 h and concentrated under *vacuum.* The residue was putted into water (25 mL) and extracted with DCM (3 x 50 mL). The combined organic layers were dried over $MgSO_4$ and evaporated. The residue was purified by flash chromatography (PE/AcOEt); 10:0 to 7:3 (*v/v*)) to afford **2** as a brown powder (71% yield); mp 158 °C; ^{1}H NMR (300 MHz, CD_2Cl_2): δ 11.09 (br s, 1H), 8.65 (d, J = 9.2 Hz, 1H), 8.42 (d, J = 1.7 Hz, 1H), 7.94 – 7.81 (m, 2H), 7.81 – 7.70 (m, 2H), 7.40 (d, J = 2.9 Hz, 1H), 7.15 (dd, J = 9.2, 2.9 Hz, 1H), 4.11 (t, J = 7.2 Hz, 2H), 3.84 (s, 3H), 3.49 (t, J = 7.2 Hz, 2H); ^{13}C NMR (75 MHz, CD_2Cl_2): δ 201.7, 168.0, 159.4, 154.8, 134.1, 133.3, 132.1, 123.1, 122.9, 120.5, 115.4, 55.7, 38.1, 33.5, 29.7

4.1.3. 4-Chloro-6-methoxyquinazoline **(4)**. A suspension of 6-methoxy-(*3H*)-quinazolin-4-one (4.4 g, 25 mmol) in $POCl_3$ (20 mL) was refluxed for 12h. The mixture was concentrated *in vacuo*, carefully hydrolyzed in ice water and alkalinized with a 28% ammonia solution. The solid was filtered off, dissolved in dichloromethane dried over $MgSO_4$ and concentrated *in vacuo*. Crude product was recrystallized from toluene to afford **4** (65% yield) as a yellow solid; mp 78-79 °C; ^{1}H (300 MHz, DMSO-d_6): δ 8.13 (s, 1H), 7.41 (d, 1H, J = 2.9 Hz), 7.32 (d, 1H, J = 9.0 Hz), 7.25 (dd, J = 9.0, 2.9 Hz, 1H), 3.95 (s, 3H).

4.1.4. (E)-2-(2-(6-Methoxyquinazolin-4-yl)vinyl)isoindoline-1,3-dione (**5**). In a sealed tube under argon atmosphere, were introduced compound **4** (0.39 g, 2.0 mmol), DMF (5 mL), triethylamine (1.2 mL, 8 mmol), palladium diacetate (22.5 mg, 0.1 mmol), tri(*o*-tolyl)-phosphine (61 mg, 0.2 mmol) and *N*-vinylphthalimide (0.52 g, 3 mmol). The mixture was stirred at 110 °C for 5 h and then hydrolyzed. The formed solid was solubilized in CH_2Cl_2, washed with water and brine, dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was recrystallized from toluene to afford **5** as a brown solid (50% yield); mp

236 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.00 (s, 1H), 8.42 (d, *J* = 15 Hz, 1H), 7.92 (d, *J* = 9.0 Hz, 1H), 7.83 – 7.82 (m, 2H), 7.80 – 7.74 (m, 2H), 7.53 (dd, *J* = 9.0, 2.8 Hz, 1H), 7.46 (d, *J* = 2.8 Hz, 1H), 6.25 (d, *J* = 15 Hz, 1H), 3.88 (s, 3H); ^{13}C NMR (75 MHz, CD_2Cl_2): δ 165.2, 162.2, 159.5, 153.6, 145.1, 132.8, 132.1, 130.2, 126.5, 127.4, 123.7, 123.3, 100.6, 95.8, 55.8

4.1.5. 2-(2-(6-Methoxyquinazolin-4-yl)ethyl)isoindoline-1,3-dione **(3)**.

Method 1. To a solution of **2** (200 mg, 0.57 mmol) in formamide (2 mL) was added ammonium formate (302 mg, 4.79 mmol) in sealed tube. The reactional mixture was stirred at 135 °C for 24 h. The mixture was putted into water (20 mL) and extracted with DCM. The combined organic layers were dried over $MgSO_4$ and evaporated. The residue was washed with methanol to give **3** as a brown powder (61% yield).

Method 2. To a solution of **5** (0.32 g, 1 mmol) in 30 mL of ethanol/THF (1/1) was added Pd/C 10%. The mixture was stirred and placed under hydrogen atmosphere at room temperature overnight. The catalyst was filtered on celite and the solvent was evaporated under reduced pressure. The obtained solid was recrystallized from toluene to afford **3** as a white solid (90% yield); mp 236 °C; ^{1}H NMR (300 MHz, CD_2Cl_2): *δ* 9.07 (s, 1H), 7.94 (d, *J* = 9.2 Hz, 1H), 7.88 – 7.82 (m, 2H), 7.80 – 7.74 (m, 2H), 7.53 (dd, *J* = 9.2, 2.7 Hz, 1H), 7.46 (d, *J* = 2.7 Hz, 1H), 4.34 – 4.14 (m, 2H), 4.00 (s, 3H), 3.69- 3.45 (m, 2H); ^{13}C NMR (75 MHz, CD_2Cl_2): *δ* 168.1, 165.5, 158.5, 152.6, 146.1, 134.0, 132.1, 130.5, 126.4, 124.9, 123.1, 101.6, 55.8, 36.3, 33.0

4.1.6. 2-(6-Methoxyquinazolin-4-yl)ethanamine **(6)**. To a solution of **3** (200 mg, 0.34 mmol) in EtOH was added hydrazine monohydrate (0.17 mL, 3.45 mmol). The mixture was stirred at room temperature for 16 h. The mixture was concentrated under *vacuum* and purified by flash chromatography (DCM/MeOH, NH_3 sat; 10:0 to 9:1 (*v/v*)) to give **6** as brown powder (97%, yield); mp 71 °C; ^{1}H NMR (300 MHz, CD_2Cl_2): *δ* 9.08 (s, 1H), 7.94 (d, *J* = 9.2 Hz, 1H), 7.55 (dd, *J* = 9.2, 2.7 Hz, 1H), 7.35 (d, *J* = 2.7 Hz, 1H), 3.98 (s, 3H), 3.40- 3.34 (m, 2H), 3.33 –

3.26 (m, 2H); ^{13}C NMR (75 MHz, CD_2Cl_2): δ 167.7, 158.2, 152.6, 145.9, 130.5, 125.9, 125.2, 102.0, 55.7, 40.2, 37.9

4.1.7. N-(2-(6-Methoxyquinazolin-4-yl)ethyl)acetamide **(7a)**.

Method 1. To a solution of **6** (64 mg, 0.32 mmol), K_2CO_3 (133 mg, 0.96 mmol) in water (3 mL) and ethyl acetate (20 mL) was added acetyl chloride (34 µL, 0.48 mmol). The mixture was stirred over 4 h. The biphasic mixture was separated, and the aqueous layer was extracted with EtOAc (3 x 20 mL). The combined organic layers were dried over $MgSO_4$, filtrated and concentrated. The residue was dissolved in DCM and precipitated by petroleum ether. The resulting precipitate was recovered and washed with PE to give **7a** as a white powder (44% yield).

Method 2. This compound was prepared from compound **8** according to the procedure described for **3** (Method 1). Recrystallization from acetonitrile gave **7a** (60% yield) as a white solid; mp 78 °C; ^{1}H NMR (300 MHz, CD_2Cl_2): δ 9.08 (s, 1H), 7.94 (d, J = 9.2 Hz, 1H), 7.52 (dd, J = 9.2 Hz, 2.8, 1H), 7.43 (d, J = 2.7 Hz, 1H), 6.47 (br s, 1H, NH), 3.99 (s, 3H), 3.89 – 3.80 (q, J = 6.3 Hz, 2H) 4.34 – 4.14 (t, J = 6.3 Hz, 2H), 1.92 (s, 3H); ^{13}C NMR (75 MHz, CD_2Cl_2): δ 169.9, 166.8, 158.5, 152.3, 145.8, 130.4, 126.4, 125.2, 101.8, 55.8, 36.7, 33.6, 23.1; HPLC: C18 column: t_R = 19.799 min, purity > 99%.

4.1.8. N-(2-(6-Methoxyquinazolin-4-yl)ethyl)propionamide **(7b)**. This compound was prepared from compound **6** according to the procedure described for **7a** (Method 1). Recrystallized from acetonitrile as a yellow solid (85% yield); mp 89-91 °C; IR: 3268 cm^{-1} (ν_{NH}), 1668 cm^{-1} (ν_{CO}); ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.08 (s, 1H), 7.94 (d, J = 9.2 Hz, 1H), 7.55 (dd, J = 9.2, 2.8 Hz, 1H), 7.40 (d, J = 2.8 Hz, 1H), 6.42 (br s, 1H), 3.99 (s, 3H), 3.86 (q, J = 6.3 Hz, 2H), 3.46 (t, J = 6.3 Hz, 2H), 2.16 (q, J = 7.5 Hz, 3H), 1.09 (t, J = 7.5 Hz, 3H); ^{13}C NMR (75 MHz, CD_2Cl_2): δ 173.5, 166.9, 158.5, 152.3, 145.8, 130.4, 126.3, 125.2, 101.8, 55.8, 36.6, 33.6, 29.6, 9.5; HPLC: C18 column: t_R = 20.382 min, purity > 99%.

4.1.9. N-(2-(6-Methoxyquinazolin-4-yl)ethyl)butyramide **(7c)**. This compound was prepared from compound **6** according to the procedure described for **7a** (Method 1). Recrystallized from acetonitrile as a yellow solid (85% yield); mp 146-148 °C; IR: 3266 cm^{-1} (ν_{NH}), 1624 cm^{-1} (ν_{CO}); ^{1}H NMR (300 MHz, CD_2Cl_2): δ 9.08 (s, 1H), 7.93 (d, *J* = 9.3 Hz, 1H), 7.55 (dd, *J* = 9.3, 2.9 Hz, 1H), 7.40 (d, *J* = 2.9 Hz, 1H), 6.44 (br s, 1H), 3.99 (s, 3H), 3.86 (q, *J* = 6.1 Hz, 2H), 3.46 (t, *J* = 6.2 Hz, 2H), 2.11 (t, *J* = 7.2 Hz, 2H), 1.60 (p, *J* = 7.5 Hz, 2H), 0.89 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, CD_2Cl_2): δ 174.7, 168.8, 160.4, 154.3, 147.7, 132.3, 128.3, 127.1, 103.8, 57.7, 40.5, 38.5, 35.6, 21.0, 15.4. HPLC: C18 column: t_R = 21.437 min, purity > 95%.

4.1.10. N-(2-(6-Methoxyquinazolin-4-yl)ethyl)cyclopropanecarboxamide **(7d)**. This compound was prepared from compound **6** according to the procedure described for **7a** (Method 1). Recrystallized from acetonitrile as a white solid (82% yield); mp 115-117 °C. IR: 3304 cm^{-1} (ν_{NH}), 1663 cm^{-1} (ν_{CO}); ^{1}H NMR (300 MHz, CD_2Cl_2): δ 9.09 (s, 1H), 7.93 (d, *J* = 9.2 Hz, 1H), 7.55 (dd, *J* = 9.2, 2.7 Hz, 1H), 7.38 (d, *J* = 2.7 Hz), 6.66 (br s, 1H), 3.98 (s, 3H), 3.88 (q, *J* = 6.2 Hz, 2H), 3.46 (t, *J* = 6.2 Hz, 2H), 1.41 – 1.31 (m, 1H), 0.92 – 0.83 (m, 2H), 0.74 – 0.65 (m, 2H); ^{13}C NMR (75 MHz, CD_2Cl_2): δ 175.2, 168.9, 160.4, 154.3, 147.7, 132.3, 128.2, 127.1, 103.8, 57.7, 38.7, 35.7, 16.4, 8.5. HPLC: C18 column: t_R = 20.382 min, purity > 99%.

4.1.11. N-(3-(2-Formamido-5-methoxyphenyl)-3-oxopropyl)acetamide **(8)**. This compound was prepared from melatonin according to the procedure described for **2**. Crude product was recrystallized from acetonitrile to afford **8** as a yellow solid (55% yield); mp 153-154 °C; IR: 3329 cm^{-1} (ν_{NH}), 1681 cm^{-1} (ν_{CO}). ^{1}H NMR (300 MHz, CD_2Cl_2): δ 11.16 (br s, 1H, NH), 8.65 (d, *J* = 9.2 Hz, 1H), 8.43 (d, *J* = 1.9 Hz, 1H), 8.45 (br s, 1H), 7.43 (d, *J* = 2.9 Hz, 1H), 7.16 (dd, *J* = 9.2, 2.9 Hz, 1H), 6.09 (br s, 1H), 3.85 (s, 3H), 3.62 (q, *J* = 5.9 Hz, 2H), 3.28 (t, *J* =

5.9 Hz, 2H), 1.92 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 203.4, 169.6, 161.9, 159.3, 154.9, 133.2, 122.9, 120.5, 115.5, 55.7, 39.8, 34.5, 23.0

4.1.12. N-(3-(2-Amino-5-methoxyphenyl)-3-oxopropyl)acetamide **(9)**. To a solution of **8** (2.65 g, 10 mmol) in EtOH was added 4 M aqueous hydrochloric acid (60 ml). The solution was refluxed for 2 h and EtOH was evaporated. The mixture was cooled to room temperature and adjusted to pH = 8 with 10 M aqueous sodium hydroxide solution and extracted with ethyl acetate. The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure to afford 9 as yellow solid (65% yield); mp 89-92 °C. IR: 3407, 3274 cm^{-1} (ν_{NH2}), 1657, 1635 cm^{-1} (ν_{CO}); ^{1}H (300 MHz, $CDCl_3$): δ 8.75 (d, J = 9.3 Hz, 1H), 7.16 (d, J = 3.0 Hz, 1H), 7.02 (dd, J = 9.3, 3.0 Hz, 1H), 6.00 (br s, 2H), 3.78 (s, 3H), 3.69 (q, J = 4.9 Hz, 2H), 3.21 (t, 2H, J = 4.9 Hz), 1.96 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 203.2, 161.5, 159.3, 153.4, 132.2, 122.7, 120.5, 117.5, 55.6, 39.8, 34.5, 23.2

4.1.13. N-(2-(3-Acetamidopropanoyl)-4-methoxyphenyl)benzamide **(10a)**. To a cold solution of **9** (0.4 g, 1.7 mmol) and TEA (0.35 mL, 2.5 mmol) in methylene chloride, was added dropwise benzoyl chloride (0.24 mL, 2.0 mmol). The solution was stirred at room temperature for 2h and water was added. The organic phase was wached with 1M NaOH and 1M HCl. The organic layer was dried over $MgSO_4$ and evaporated. The obtained solid was recrystallized from acetonitrile to afford **10a** (67% yield) as a yellow solid; mp 160-162 °C; IR: 3355, 3342 cm^{-1} (ν_{NH}), 1672, 1643, 1635 cm^{-1} (ν_{CO}); ^{1}H NMR (300 MHz, $CDCl_3$): δ 12.29 (br s, 1H), 8.89 (dd, J = 9.3, 1.5 Hz, 1H), 8.05-8.03 (m, 2H), 7.58-7.51 (m, 3H), 7.41 (m, 1H), 7.21-7.18 (m, 1H), 6.24 (m, 1H), 3.85 (m, 3H), 3.69 (q, J = 5.7 Hz, 2H), 3.32 (t, J = 5.1 Hz, 2H), 1.99 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 203.7, 170.3, 165.7, 154.6, 134.9, 131.9, 128.8, 127.3, 122.5, 122.5, 121.2, 115.5, 55.7, 39.7, 34.5, 23.3

4.1.14. N-(2-(3-Acetamidopropanoyl)-4-methoxyphenyl)-3-methoxybenzamide **(10b)**. This compound was prepared from **9** according to the procedure described for **10a**.

Recrystallization from acetonitrile gave **10b** (70% yield) as a yellow solid; mp 148-153 °C; IR: 3366, 3215 cm^{-1} (ν_{NH}), 1662, 1652, 1638 cm^{-1} (ν_{CO}); ^{1}H NMR (300 MHz, $CDCl_3$): δ 12.26 (br s, 1H), 8.86 (d, 1H, *J* = 9.2 Hz), 7.59-7.57 (m, 2H), 7.45-7.39 (m, 2H), 7.18 (dd, *J* = 9.2, 2.8 Hz, 1H), 7.10 (m, 1H), 6.28 (m, 1H), 3.90 (s, 3H), 3.85 (s, 3H), 3.68 (q, *J* = 5.8 Hz, 2H), 3.30 (t, *J* = 5.7 Hz, 2H), 1.98 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 203.6, 170.4, 165.5, 159.9, 154.6, 136.4, 134.8, 129.8, 122.6, 122.4, 121.1, 119.1, 118.1, 115.5, 112.7, 55.7, 55.4, 39.7, 34.5, 23.3

4.1.15. N-(2-(3-Acetamidopropanoyl)-4-methoxyphenyl)-2-cyclopropylacetamide **(10c)**. This compound was prepared from **9** according to the procedure described for **10a**. Recrystallization from acetonitrile afford **10c** (70% yield) as a yellow solid; mp 122-125 °C; IR: 1669, 1658, 1648 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 11.24 (br s, 1H), 8.67 (d, *J* = 9.5 Hz, 1H), 8.45 (br s, 1H), 7.38 (d, *J* = 2.9 Hz, 1H), 7.14 (dd, *J* = 9.2, 2.8 Hz, 1H), 3.84 (s, 3H), 3.67 (q, *J* = 5.8 Hz, 2H), 3.27 (t, *J* = 5.8 Hz, 2H), 1.35 (m, 2H), 1.12-0.70 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 203.5, 173.8, 159.5, 154.9, 133.2, 123.2, 122.7, 120.7, 115.6, 55.7, 39.8, 34.5, 29.7, 14.7, 7.25, 7.25

4.1.16. N-(2-(6-Methoxy-2-phenylquinazolin-4-yl)ethyl)acetamide **(11a)**. This compound was prepared from compound **10a** according to the procedure described for **7a** (Method 1). Recrystallized from acetonitrile as a white solid (58% yield); mp 157-158 °C; ^{1}H NMR (300 MHz, CD_2Cl_2): δ 8.61 (m, 2H), 8.00 (d, *J* = 9.2 Hz, 1H), 7.64-7.47 (m, 4H), 7.40 (d, *J* = 2.8 Hz, 1H), 6.43 (br s, 1H), 4.07-3.88 (m, 5H), 3.60-3.45 (t, *J* = 6.2 Hz, 2H), 1.92 (s, 3H); ^{13}C NMR (75 MHz, CD_2Cl_2): δ 169.9, 166.9, 158.3, 157.6, 146.5, 138.3, 130.6, 130.1, 128.5, 127.9, 126.4, 123.6, 102.0, 55.8, 36.6, 33.7, 23.1; HPLC: C18 column: t_R = 26.753 min, purity > 99%.

4.1.17. N-(2-(6-Methoxy-2-(3-methoxyphenyl)quinazolin-4-yl)ethyl)acetamide **(11b)**. This compound was prepared from compound **10b** according to the procedure described for **7a**

(Method 1). Recrystallized from acetonitrile as a white solid (54% yield); mp 151-152 °C; ^{1}H NMR (300 MHz, CD_2Cl_2): δ 8.24-8.15 (m, 2H), 7.99 (d, J = 9.2 Hz, 1H), 7.55 (dd, J = 9.2, 2.8 Hz, 1H), 7.46 (t, J = 8.0 Hz, 1H), 7.38 (d, J = 2.8 Hz, 1H), 7.06 (ddd, J = 8.0, 2.7, 0.9 Hz, 1H), 6.42 (br s, 1H), 4.00 (s, 3H), 3.98-3.92 (m, 5H), 3.51 (t, J = 6.3 Hz, 2H), 1.92 (s, 3H); ^{13}C NMR (75 MHz, CD_2Cl_2): δ 169.9, 166.8, 160.1, 158.3, 157.4, 146.4, 139.9, 130.6, 129.5, 126.4, 123.7, 120.4, 116.0, 112.9, 102.0, 55.8, 55.3, 36.6, 33.7, 23.1; HPLC: C18 column: t_R = 27.341 min, purity > 99%.

4.1.18. N-(2-(2-(Cyclopropylmethyl)-6-methoxyquinazolin-4-yl)ethyl)acetamide **(11c)**. This compound was prepared from compound **10c** according to the procedure described for **7a** (Method 1). Recrystallized from acetonitrile as a white solid (85% yield); mp 114-115 °C; IR: 3318 cm^{-1} (ν_{NH}), 1638 cm^{-1} (ν_{CO}); ^{1}H (300 MHz, DMSO-d_6): δ 7.90 (d, J = 9.2 Hz, 1H), 7.52 (dd, J = 9.2, 2.8 Hz, 1H), 7.29 (d, J = 2.8 Hz, 1H), 6.66 (br s, 1H), 3.96 (s, 3H), 3.93 (m, 2H), 3.46 (t, J = 6.0 Hz, 2H), 2.98 (d, J = 7.1 Hz, 2H), 1.96 (s, 3H), 1.34 (m, 1H), 0.60 (m, 4H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 170.29, 166.73, 163.80, 157.89, 146.07, 129.96, 126.41, 123.05, 101.57, 55.76, 44.07, 36.65, 33.39, 23.40, 10.46, 4.59, 4.59; HPLC: C18 column: t_R = 20. 544 min, purity > 95%.

4.1.19. N-(2-((6-Methoxyquinazolin-4-yl)amino)ethyl)acetamide **(12)**. To a solution of **4** (0.5 g, 2.5 mmol) in THF was added Et_3N (0.52 mL, 3.76 mmol) and *N*-(2-aminoethyl)acetamide (0.25 g, 2.5 mmol). The mixture was stirred at 30 °C for 1h, cooled to room temperature and concentrated *in vacuo*. The residue was suspended in water and extracted with EtOAc. The organic phase was dried over $MgSO_4$ and concentrated by evaporation *in vacuo.* Solid was recrystallized from toluene to afford **12**. Recrystallized from acetonitrile as a white solid (55% yield); mp 176-178 °C; IR: 3304 cm^{-1} (ν_{NH}), 3195 cm^{-1} (ν_{NH}), 1663 cm^{-1} (ν_{CO}); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.52 (s, 1H), 7.88 (br s, 1H), 7.74 (d, J = 9.1 Hz, 1H), 7.36 (dd, J = 9.1, 2.6 Hz, 1H), 7.30 (d, J = 2.6 Hz, 1H), 6.97 (br s, 1H), 4.56 (br s, 1H), 3.94 (s, 3H), 3.82-3.72 (m,

2H), 3.70-3.61 (m, 2H), 2.04 (s, 3H); ^{13}C NMR (CDCl3): δ 172.9, 159.3, 157.8, 152.9, 143.9, 129.3, 124.2, 115.4, 100.6, 55.8, 43.7, 39.9, 23.3; HPLC: C18 column: t_R = 20.382 min, purity > 99%.

4.1.20. N-Ethyl-4-(6-methoxyquinazolin-4-yl)piperazine-1-carboxamide **(13)**. This compound was prepared from compound **4** according to the procedure described for **12**. Recrystallized from toluene as a white solid (75% yield); mp 145-147 °C; IR: 3255 cm^{-1} (ν_{NH}), 1643 cm^{-1} (ν_{CO}); ^{1}H NMR (300 MHz, CD_2Cl_2): δ 8.66 (s, 1H), 7.84 (d, J = 9.1 Hz, 1H), 7.45 (dd, J = 9.1, 2.8 Hz, 1H), 7.20 (d, J = 2.8 Hz, 1H) 4.60 (br s, 1H), 3.94 (s, 3H), 3.64 - 3.56 (m, 4H), 3.75-3.70 (m, 4H), 3.33 - 3.23 (m, 2H), 1.17 (t, J = 7.2 Hz, 3H) ; ^{13}C NMR (75 MHz, CD_2Cl_2): δ 164.3, 157.6, 157.0, 152.1, 147.3, 130.2, 124.2, 117.6, 103.4, 55.6, 49.3, 43.4, 35.7, 15.3; HPLC: C18 column: t_R = 20.382 min, purity > 99%.

4.1.21. Cyclopropyl-(4-(6-methoxyquinazolin-4-yl)piperazin-1-yl)methanone **(14)**. This compound was prepared from compound **4** according to the procedure described for **12**. Recrystallized from toluene as a white solid (75% yield); mp 145-147 °C; IR: 3255 cm^{-1} (ν_{NH}), 1643 cm^{-1} (ν_{CO}); ^{1}H NMR (300 MHz, CD_2Cl_2): δ 8.68 (s, 1H), 7.86 (d, *J* = 9.2 Hz, 1H), 7.45 (dd, *J* = 9.2, 2.8 Hz, 1H), 7.21 (d, *J* = 2.8 Hz, 1H), 4.00-3.65 (m, 11H), 1.83 (m, 1H), 1.02-0.93 (m, 2H), 0.86 – 0.77 (m, 2H); ^{13}C NMR (75 MHz, CD_2Cl_2): δ 172.1, 164.3, 157.1, 152.1, 147.4, 130.2, 124.2, 117.6, 103.4, 55.6, 49.8, 49.3, 45.0, 41.7, 29.7, 10.9, 7.1; HPLC: C18 column: t_R = 20.382 min, purity > 99%.

4.1.22. 1-Chloro-7-methoxyphthalazine **(16)**. A suspension of 7-methoxyphthalazin-(2*H*)-one (**15)** (1.0 g, 5.7 mmol) in $POCl_3$ (3 mL) was refluxed for 1h. After cooling, the reaction mixture was carefully hydrolyzed in crunched ice and alkalinized with a 15% NaOH solution. The solid was filtered off, dissolved in dichloromethane dried over $MgSO_4$ and concentrated *in vacuo*. Crude product **16** (86% yield) was used without further purification; mp 139-141

°C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.30 (s, 1H), 7.90 (d, J = 8.6 Hz, 1H), 7.60 (dd, J = 8.6, 2.5 Hz, 1H), 7.50 (d, J = 2.5 Hz, 1H), 4.05 (s, 3H).

4.1.23. Ethyl 2-cyano-2-(7-methoxyphthalazin-1-(2H)-ylidene)acetate **(17)**. In anhydrous THF (10 mL), a solution of ethyl cyanocetate (0.66 mL, 6.2 mmol) was added to a mixture of NaH (60% in mineral oil) (250 mg, 6.2 mmol). The mixture was stirred for 30 min at r.t., then a solution of **15** (0.8 g, 4.1 mmol) in anhydrous THF (10 mL) was added. The reaction mixture was heated at reflux for 12 h, cooled and quenched with water. The solid was filtered, washed with water, dried. Recrystallization from acetonitrile gave **17** as beige powder (80% yield); mp 185-187 °C; IR 2250 cm^{-1} (ν_{CN}), 1650 cm^{-1} (ν_{CO}); ^{1}H NMR (300 MHz, $CDCl_3$): δ 13.55 (br s, 1H), 9.00 (d, J = 2.6 Hz, 1H), 8.30 (s, 1H), 7.70 (d, J = 8.4 Hz, 1H), 7.45 (dd J = 8.4, 2.6 Hz, 1H,), 4.25 (q, J = 7.0 Hz, 2H), 4.00 (s, 3H), 1.40 (t, J = 7.0 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.3, 165.2, 161.5, 139.1, 135.3, 130.0, 128.4, 116.2, 113.0, 105.9, 70.2, 61.4, 56.1, 13.5

4.1.24. 2-(7-Methoxyphthalazin-1-yl)acetonitrile **(18)**. A mixture of **17** (0.46 g, 1.7 mmol) in 5M HCl (80 ml) was refluxed until complete dissolution. The solution was refluxed for an additional 2h then ethanol was evaporated. The mixture was cooled to room temperature and adjusted to pH 8 with an10 M NaOH solution and extracted with ethyl acetate. The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure to afford after recrystallization in toluene **18** as beige solid (60%, yield) ; mp 165-166 °C; IR 2250 cm^{-1} (ν_{CN}); ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.40 (s, 1H), 7.96 (d, J = 8.9 Hz, 1H), 7.58 (dd, J = 8.9, 2.3 Hz, 1H), 7.29 (m, 1H), 4.45 (s, 2H), 4.05 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.1, 129.3, 129.0, 126.9, 125.2, 121.0, 115.9, 105.4, 101.4, 56.1, 23.5

4.1.25. 2-(7-Methoxyphthalazin-1-yl)ethanamine **(19)**. A NH_3-saturated solution of **18** (2.7 g, 8.5 mmol) in methanol (50 mL) was hydrogenated over Raney nickel under pressure (3 bars) at room temperature for 48 h. After filtration and evaporation, the residual oil was dissolved

in a mixture of toluene/methanol in the presence of 10% Pd/C. The mixture was refluxed for 5h, filtered and evaporated. The residue was purified by flash chromatography (CH_2Cl_2/MeOH); 10:0 to 0:10 (*v/v*)) to afford **19** as a brown oil (70% yield); IR 3250 cm^{-1} (ν_{NH2}); ^1H NMR (300 MHz, $CDCl_3$): δ 9.30 (s, 1H), 7.85 (d, *J* = 8.8 Hz, 1H), 7.45 (dd, *J* = 8.8, 2.1 Hz, 1H), 7.30 (d, *J* = 2.1 Hz, 1H), 4.00 (s, 3H); 3.50 (m, 2H), 3.40 (t, *J* = 5.5 Hz, 2H), 3.00 (br s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.8, 163.3, 156.2, 149.7, 128.6, 127.1, 125.6, 122.8, 101.2, 55.0, 40.3, 35.7

4.1.26. N-(2-(7-Methoxyphthalazin-1-yl)ethyl)acetamide **(20a)**. To a solution of **19** (0.5 g, 2.5 mmol), K_2CO_3 (1.0 g, 14.7 mmol) in water (20 mL) and ethyl acetate (40 mL) was added acetyl chloride (0.35 mL, 5 mmol). The mixture was stirred for 2h. The biphasic mixture was separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtrated and concentrated. The residue was first purified by flash chromatography (CH_2Cl_2/MeOH; 9/1) and recrystallized from toluene to give **20a** as a white powder (40% yield); mp 160-163 °C; IR: 3250 cm^{-1} (ν_{NH}), 1665 cm^{-1} (ν_{CO}); ^1H NMR (300 MHZ, $CDCl_3$): δ 9.30 (s, 1H), 7.85 (d, *J* = 8.8 Hz, 1H), 7.50 (dd, *J* = 8.8, 2.4 Hz, 2H), 7.40 (d, *J* = 2.4 Hz, 1H), 6.70 (br s, 1H), 4.05 (s, 3H), 3.95 (q, *J* = 6.2 Hz, 2H), 3.50 (t, *J* = 6.2 Hz, 2H), 1.98 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.8, 162.3, 157.2, 149.6, 128.8, 128.1, 124.6, 121.8, 102.2, 56.0, 37.3, 32.7, 23.3; HPLC: C18 column: t_R = 17.266 min, purity > 99%.

4.1.27. N-(2-(7-Methoxyphthalazin-1-yl)ethyl)propionamide **(20b)**. This compound was prepared from compound **19** according to the procedure described for **20a**. Recrystallized from toluene as a white solid (55% yield); mp 137-139 °C; IR: 3250 cm^{-1} (ν_{NH}), 1660 cm^{-1} (ν_{CO}); ^1H NMR (300 MHz, $CDCl_3$): δ 9.30 (s, 1H), 7.85 (d, *J* = 8.8 Hz, 1H), 7.50 (dd, *J* = 8.8, 2.3 Hz, 1H), 7.40 (d, *J* = 2.3 Hz, 1H), 6.60 (br s, 1H), 4.05 (s, 3H), 3.95 (q, *J* = 6.2 Hz, 2H), 3.50 (t, *J* = 6.2 Hz, 2H), 2.20 (q, *J* = 7.5 Hz, 2H), 1.10 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (75

MHz, $CDCl_3$): δ 174.2, 162.7, 157.2, 149.6, 128.8, 128.1, 124.5, 121.8, 102.1, 56.0, 36.9, 32.6, 29.8, 9.9; HPLC: C18 column: t_R = 17.813 min, purity > 99%.

4.1.28. N-(2-(7-methoxyphthalazin-1-yl)ethyl)butyramide **(20c)**. This compound was prepared from compound **19** according to the procedure described for **20a**. Recrystallized from toluene as a white solid (50% yield); mp 127-129 °C; IR: 3250 cm^{-1} (ν_{NH}), 1655 cm^{-1} (ν_{CO}); ^1H NMR (300 MHz, $CDCl_3$): δ 9.40 (s, 1H), 7.88 (d, J = 8.9 Hz, 1H), 7.51 (dd, J = 8.8, 2.4 Hz, 1H), 7.43 (m, 1H), 6.66 (br s, 1H), 4.05 (s, 3H), 3.96 (q, J = 6.1 Hz, 2H), 3.53 (t, J = 6.3 Hz, 2H), 2.15 (t, J = 7.4 Hz, 2H), 1.64 (m, 2H), 0.90 (t, J = 7.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.4, 162.7, 157.2, 149.6, 128.8, 128.1, 124.5, 121.8, 102.1, 56.0, 38.8, 36.7, 32.6, 19.2, 13.7; HPLC: C18 column: t_R = 18.688 min, purity > 99%.

4.1.29. N-(2-(7-Methoxyphthalazin-1-yl)ethyl)cyclopropanecarboxamide **(20d)**. This compound was prepared from compound **19** according to the procedure described for **20a**. Recrystallized from toluene as a white solid (50% yield); mp 162-164 °C; IR: 3260 cm^{-1} (ν_{NH}), 1655 cm^{-1} (ν_{CO})^1H NMR (300 MHz, $CDCl_3$): δ 9.26 (s, 1H), 7.86 (d, J = 8.8 Hz, 1H), 7.49 (dd, J = 8.8, 2.3 Hz, 1H), 7.41 (m, 1H), 7.03 (m, 1H), 4.02 (s, 3H), 3.96 (q, J = 6.0 Hz, 2H), 3.52 (t, J = 6.3 Hz, 2H), 1.43-1.35 (m, 1H), 0.95-0.90 (m, 2H), 0.72-0.66 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.9, 162.7, 157.3, 149.6, 128.8, 128.1, 124.4, 121.8, 102.2, 56.0, 37.1, 32.7, 14.7, 7.0; HPLC: C18 column: t_R = 18.179 min, purity > 99%.

4.2. Binding studies

4.2.1. Reagents and chemicals

2-[125I]Iodomelatonin (2200 Ci/mmol) was purchased from NEN (Boston, MA). Other drugs and chemicals were purchased from Sigma–Aldrich Sigma-Aldrich (Saint Quentin, France) and used without further purification.

4.2.2. Cell Culture

HEK (provided by A.D. Strosberg, Paris, France) and CHO cell lines stably expressing the human melatonin MT_1 or MT_2 receptors were grown in DMEM medium supplemented with 10% fetal calf serum, 2 mM glutamine, 100 IU/mL penicillin and 100 mg/mL streptomycin. Grown at confluence at 37 °C (95%O_2/5%CO_2), they were harvested in PBS containing EDTA 2 mM and centrifuged at 1000 x *g* for 5 min (4 °C). The resulting pellet was suspended in TRIS 5mM (pH 7.5), containing EDTA 2mM and homogenized using a Kinematica polytron. The homogenate was then centrifuged (95000 *g*, 30 min, 4 °C) and the resulting pellet suspended in 75 mM TRIS (pH 7.5), 12.5 mM $MgCl_2$ and 2 mM EDTA. Aliquots of membrane preparations were stored at -80 °C until use.

4.2.3. Binding assays for MT_1 and MT_2 receptors subtypes

2-[^{125}I] Iodomelatonin competition binding assay conditions were essentially as previously described [21]. Briefly, binding was initiated by addition of membrane preparations from transfected CHO cells stably expressing the human melatonin MT_1 or MT_2 diluted in binding buffer (50 mM Tris–HCl buffer, pH 7.4, containing 5 mM $MgCl_2$) to 2-[^{125}I]iodomelatonin (20 pM for MT_1 and MT_2 receptors expressed in CHO cells) and the tested drug. Non-specific binding was defined in the presence of 1 μM of melatonin. After 120 min incubation at 37 °C, reaction was stopped by rapid filtration through GF/B filters presoaked in 0.5% (v/v) polyethylenimine. Filters were washed three times with 1 mL of ice-cold 50 mM Tris–HCl buffer (pH 7.4).

Data from the dose–response curves (seven concentrations in duplicate) were analysed using the program PRISM (Graph Pad Software Inc., San Diego, CA) to yield IC_{50} (inhibitory concentration 50). Affinities are expressed as pKi (pKi = -Log10 (Ki)) with Ki = IC_{50}/1 + ([L]/KD), where [L] is the concentration of radioligand used in the assay and KD, the dissociation constant of the radioligand characterizing the membrane preparation [22].

[^{35}S] GTPγS binding assay was performed according to published methodology [21]. Briefly, membranes from transfected CHO cells expressing MT_1 or MT_2 receptor subtype and compounds were diluted in binding buffer (20 mM HEPES, pH 7.4,100mMNaCl, 3 mM GDP, 3 mM $MgCl_2$, and 20 mg/mL saponin). Incubation was started by the addition of 0.2 nM [^{35}S]GTPγS to membranes (20 mg/mL) and drugs, and further followed for 1h at room temperature. For experiments with antagonists, membranes were pre-incubated with both the melatonin (3 nM) and the antagonist for 30 min prior the addition of [^{35}S]GTPγS. Non-specific binding was defined using cold GTPγS (10 mM). Reaction was stopped by rapid filtration through GF/B filters followed by three successive washes with ice cold buffer.

Usual levels of [^{35}S]GTPγS binding (expressed in dpm) were for CHO-MT_1 or MT_2 membranes: 2000 for basal activity, 8000 in the presence of melatonin 1 mM and 180 in the presence of GTPγS 10 mM which defined the non-specific binding. Data from the dose-response curves (7 concentrations in duplicate) were analyzed by using the program PRISM (Graph Pad Software Inc., San Diego, CA) to yield EC_{50} (Effective concentration 50%) and E_{max} (maximal effect) for agonists. Antagonist potencies are expressed as $K_B = IC_{50}/1 + ([Ago]/EC_{50}$ ago), where IC_{50} is the inhibitory concentration of antagonist that gives 50% inhibition of [^{35}S] GTPγS binding in the presence of a fixed concentration of melatonin ([Ago]) and EC_{50} ago is the EC_{50} of the molecule when tested alone. I_{max} (maximal inhibitory effect) was expressed as a percentage of that observed with melatonin at 3 nM for MT_2 receptor.

Serotonin 5-HT_{2C} binding assay was determined according to reported tests [23].

4.2.4. cLogP and cytotoxicity

The *n*-octanol-water partition coefficients, which are well established measures of the compounds' hydrophilicity, were calculated for final prepared compounds by use of theoretical procedures previously [24]. Numerical values of the partition coefficients were

obtained as follows using using Data Warrior software: the logP value, which is the logarithm of the partition coefficient of a compound between n-octanol and water $\log(C_{octanol}/C_{water})$, is calculated as increment system the contributions of every atom based on its atom type. Cytotoxic activities towards standard cancer cell line Caco-2 was performed using the same procedure as was previously described [25].

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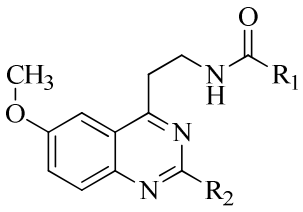
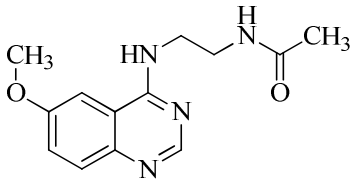
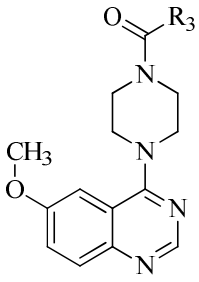
Table 1. MT_1/MT_2 binding affinities of quinazoline derivatives



7a-7d; 11a-11c



12

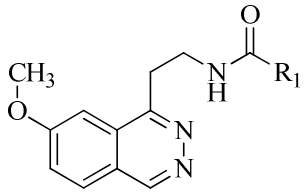


13-14

Cpd.	***R_1***	***R_2***	***R_3***	***Ki (MT_1)***	***Ki (MT_2)***
AGM	-	-	-	0.12 *[0.12;0.12] (2)*	0.21 *[0.08;0.57] (2)*
7a	CH_3	H	-	230 *[57;49] (2)*	115 *[28;39] (2)*
7b	C_2H_5	H	-	210 *[34;54] (2)*	41.1 *[8;11] (2)*
7c	C_3H_7	H	-	320 *[27;63] (2)*	41 *[9;8] (2)*
7d	c-C_3H_5	H	-	549 *[75;86] (2)*	398 *[84;47] (2)*
11a	CH_3	C_6H_5	-	896 *[115;95] (2)*	15.6 *[3;8] (2)*
11b	CH_3	C_6H_4-*m*-OCH_3	-	500 *[95;59] (2)*	46 *[12;8] (2)*
11c	CH_3	CH_2-*c*-C_3H_5	-	22.1 *[5;3] (2)*	25.9 *[7;10] (2)*
12	CH_3	H	-	$>10^{-5}$	$>10^{-5}$
13	-	H	NHC_2H_5	$>10^{-5}$	2800 *[454;472] (2)*
14	-	H	*c*-C_3H_5	$>10^{-5}$	3295 *[624;437] (2)*

*K*i (nM) values are geometric mean values (with 95% confidence limits shown in brackets) of at least n separate experiments performed in duplicate.

Table 2. MT_1/MT_2 binding affinities of the phthalazine derivatives



Cpd.	***R_1***	***Ki (MT_1)***	***Ki (MT_2)***
AGM	-	0.12 *[0.12;0.12] (2)*	0.21 *[0.08;0.57] (2)*
20a	CH_3	2110 *[548;624] (2)*	799 *[137;98] (2)*
20b	C_2H_5	1470 *[235;354] (2)*	414 *[39;101] (2)*
20c	C_3H_7	1140 *[322;147] (2)*	108 *[65;33] (2)*
20d	c-C_3H_5	3020 *[745;635] (2)*	1370 *[128;69] (2)*

*K*i (nM) values are geometric mean values (with 95% confidence limits shown in brackets) of at least n separate experiments performed in duplicate.

Table 3. Intrinsic activity of most interesting synthesized quinazolines and phthalazines.

Cpd.	*h-MT₁*		*h-MT₂*	
	EC_{50} (nM) *[I95] (n)*	**E_{max} (%)** ± *ESM (n)*	**EC_{50} (nM)** ± *[I95] (n)*	**E_{max} (%)** ± *ESM (n)*
AGM	1.56 *[1.12;2.00] (4)*	99± 6 *(4)*	0.21 *[0.13;0.30] (3)*	91± 7 *(3)*
7a	2600 *[754;694] (2)*	83 ± 6 *(2)*	835 *[47;123] (2)*	99 ± 3 *(2)*
7b	432 *[38;64] (2)*	96 ± 7 *(2)*	238 *[54;39] (2)*	102 ± 8 *(2)*
7c	652 *[59;81] (2)*	89 ± 3 *(2)*	219 *[27;14] (2)*	117 ± 3 *(2)*
11a	$>10^{-5}$	nd	237 *[25;43] (2)*	91± 3 *(2)*
11b	321 *[33;67] (2)*	72 ± 3 (2)	543 *[68;84] (2)*	48 ± 7 *(2)*
11c	429 *[37;71] (2)*	88 ± 5 *(2)*	238 *[55;28] (2)*	105 ± 4 *(2)*
20a	$>10^{-5}$	nd	428 *[27;33] (2)*	65 ± 7 *(2)*
20b	$>10^{-5}$	nd	327 *[77;63] (2)*	90 ± 3 *(2)*
20c	$>10^{-5}$	nd	89 *[11;8] (2)*	89 ± 7 *(2)*
20d	$>10^{-5}$	nd	1253 *[456;389] (2)*	68 ± 11 *(2)*

nd: not determined.

EC_{50} values are geometric mean values of (n) experiments.

Emax values are arithmetic mean ± S.E.M.

Table 4. ClogP results of quinazoline and phthalazine derivatives

Cpd.	*ClogP (Pipeline Pilot)*
AGM	2.62
7a	1.24
7b	1.69
7c	2.15
7d	1.56
11a	2.88
11b	2.81
11c	1.97
12	0.83
13	1.67
14	1.85
20a	0.85
20b	1.31
20c	1.76
20d	1.17

ClogP calculated by Pipeline Pilot – Biovia dassault Systems; values are geometric mean values of 3 experiments.

Table 5. Caco2 and bioavailability data of prepared quinazoline and phthalazine derivatives

Cpd.	*Bioavailability (R/H)*	*Caco2 %*
AGM	13/11	100
7a	92/97	100
7c	69/36	nd
11a	2/7	100
11b	nd	nd
11c	23/54	100
20a	nd	2
20b	54/46	100
20c	33/36	100
20d	nd	nd

nd: not determined; R: Rodent; H: human; values are geometric mean values of 2 experiments.

Figure 1. Aza-agomelatine derivatives

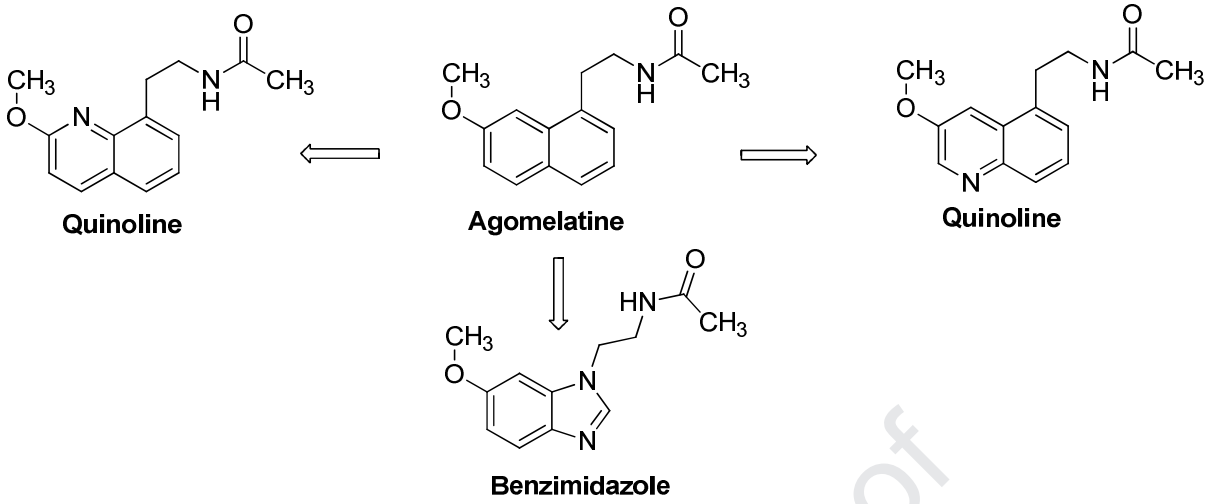
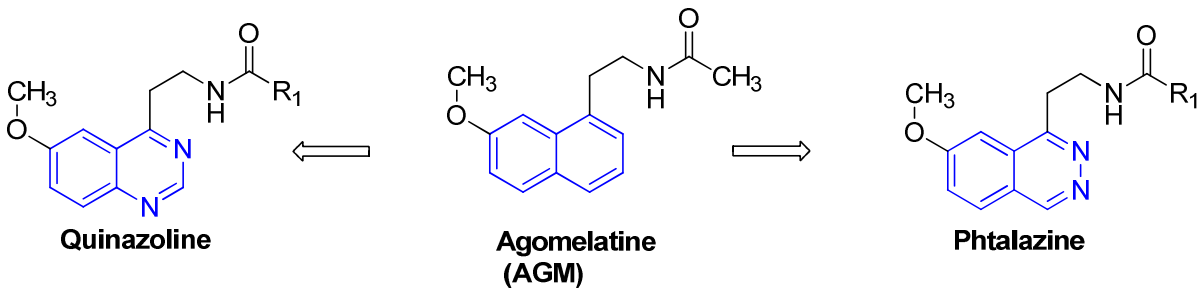
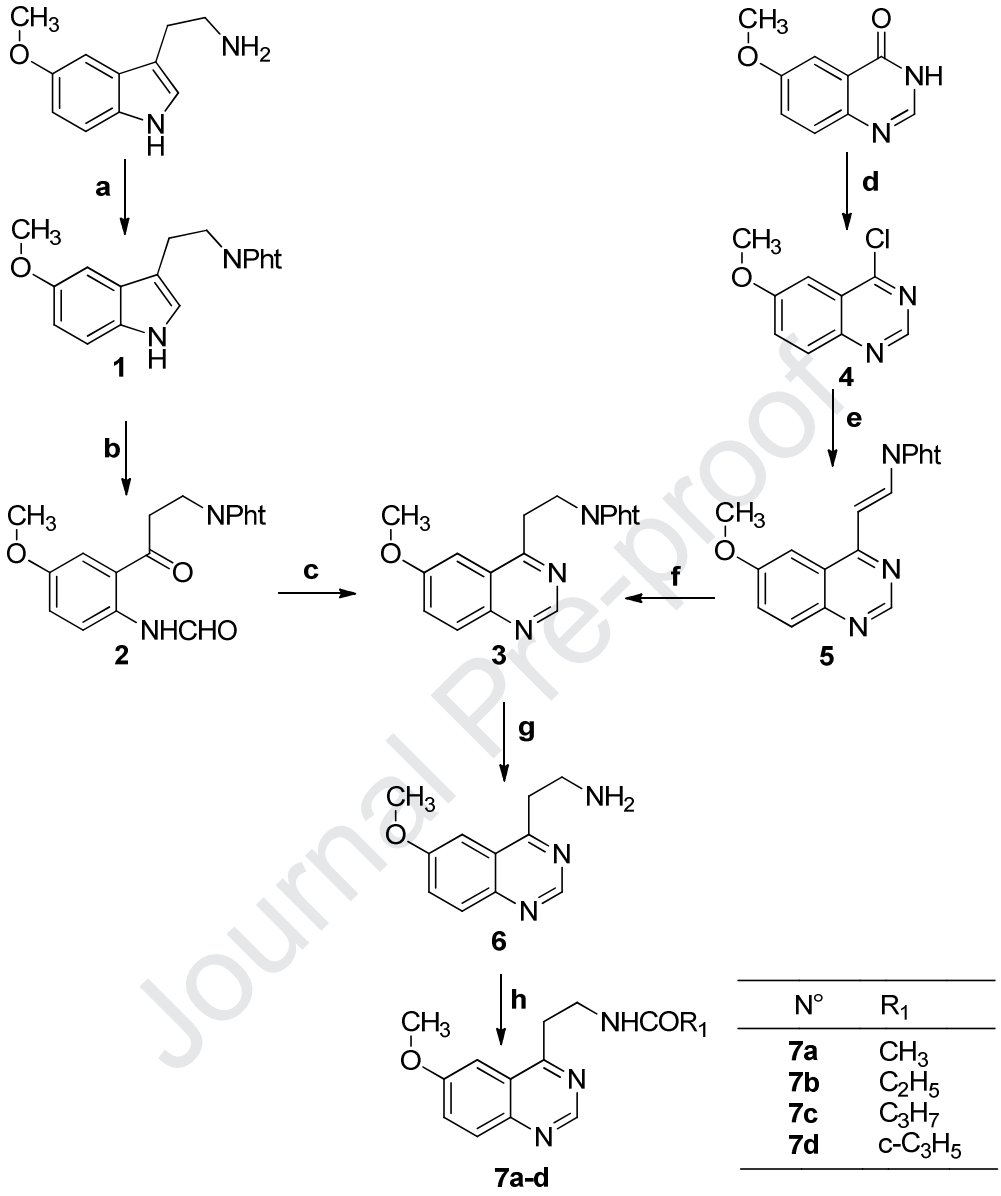


Figure 2. Design of novel Aza-agomelatine derivatives



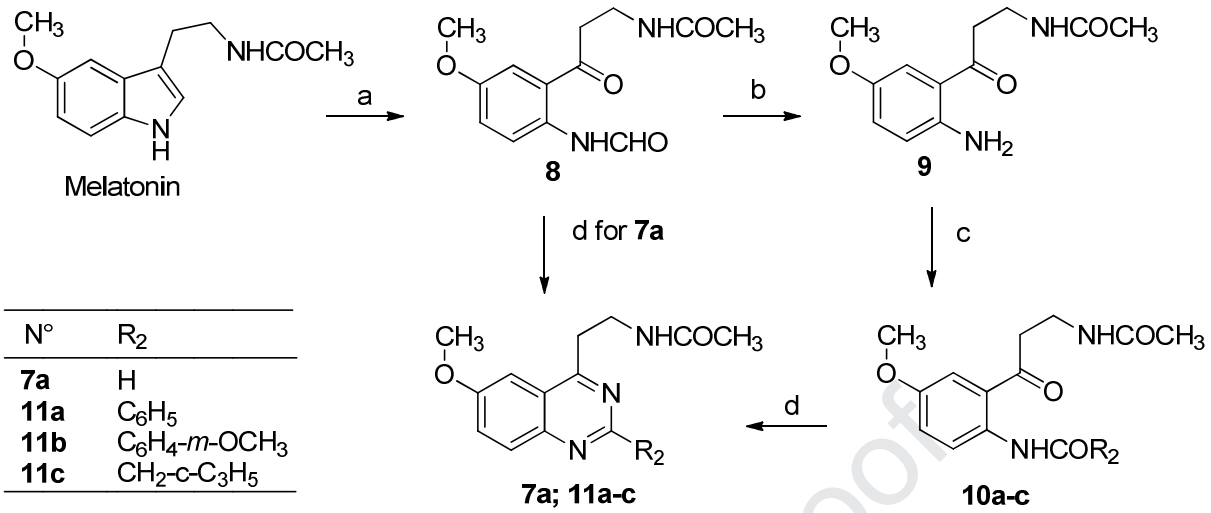
Scheme 1[a]**.** Synthesis of 4,6-disubstituted quinazolines **7a-d.**



N°	R_1
7a	CH_3
7b	C_2H_5
7c	C_3H_7
7d	c-C_3H_5

[a]*Reagents and conditions*: a) Phtalic anhydride, TEA, toluene, reflux, 87%; b) $NaIO_4$, MeOH, 80°C, 71%; c) Ammonium formate, formamide, 135°C, 61%; d) $POCl_3$, reflux, 65%; e) *N*-Vinylphthalimide, $Pd(OAc)_2$, tris-(*o*-tolyl) phosphine, DMF, 110°C, 50%; f) H_2, Pd/C, EtOH-THF, rt, 90%; g) H_2N-$NH_2.H_2O$, EtOH, rt, 97%; h) R_1COCl, K_2CO_3, EtOAc-H_2O, 44-82%.

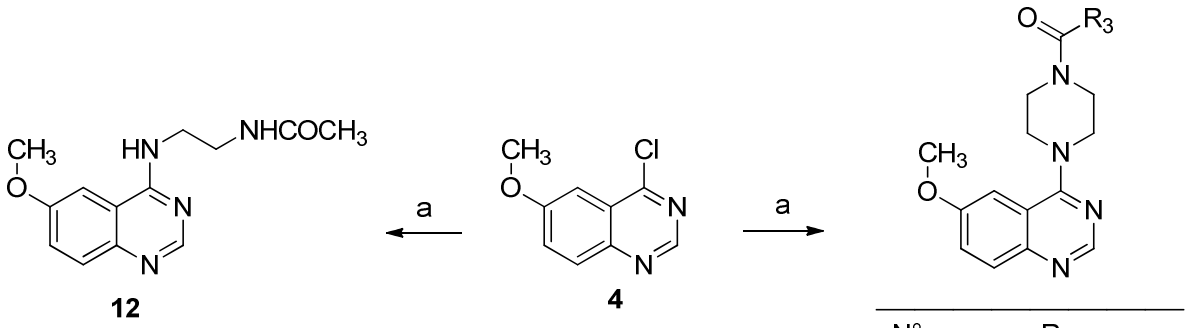
Scheme 2[a]**.** Synthesis of 2,4,6- trisubstituted quinazoline derivatives **11a-d**



N°	R_2
7a	H
11a	C_6H_5
11b	C_6H_4-*m*-OCH_3
11c	CH_2-c-C_3H_5

[a]*Reagents and conditions:* a) $NaIO_4$, MeOH, 80°C, 55%; b) 4M HCl, EtOH, reflux, 85%; c) R_2COCl, Et_3N, CH_2Cl_2, 67-70%; d) Ammonium formate, formamide, 135°C, 54-85%.

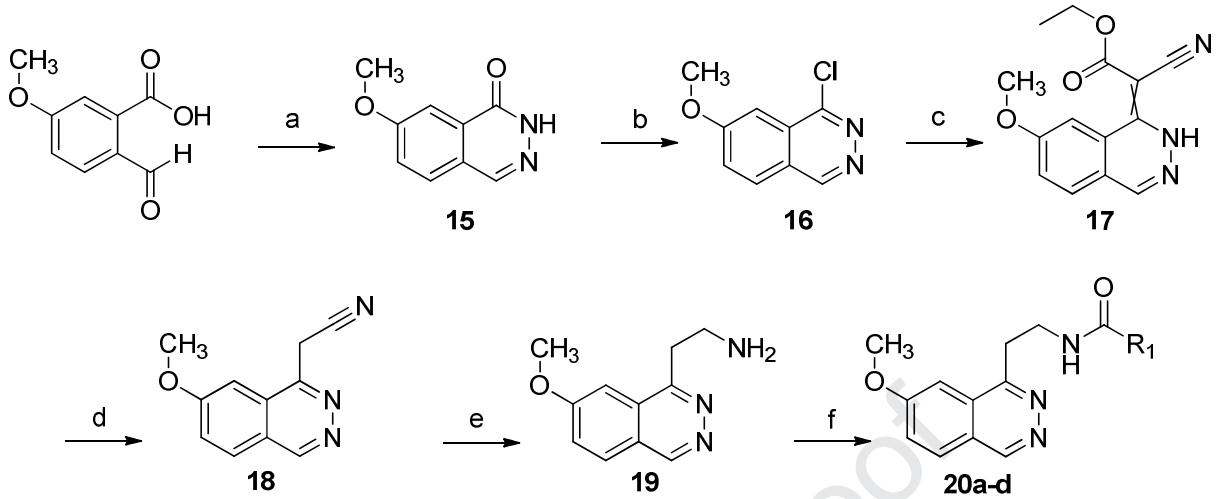
Scheme 3[a]**.** Synthesis of 4,6-disubstituted quinazoline derivatives **12-14**



N°	R_3
13	NH-C_2H_5
14	*c*-C_3H_5

[a]*Reagents and conditions:* a) Appropriate amine, THF, Et_3N, 30°C, 55-75%.

Scheme 4[a]**.** Synthesis of phthalazine derivatives **20a-d**



N°	R_1
20a	CH_3
20b	C_2H_5
20c	C_3H_7
20d	*c*-C_3H_5

[a]*Reagents and conditions:* a) $NH_2NH_2.H_2O$, EtOH, reflux, 65%; b) $POCl_3$, reflux, 86%; c) Ethyl cyanoacetate, NaH, anhydrous THF, 80%; d) 5M HCl, reflux, 60%; e) H_2, Raney Ni, NH_3(g), rt, 70%; f) R_1COCl, K_2CO_3, EtOAc/H_2O, 40-55%.

Highlights

- New quinazoline and phthalazine analogues of agomelatine were prepared
- Obtained compounds showed good melatonergic affinity and no 5-HT_{2C} affinity
- Quinazoline **11c** showed the most interesting results of this series
- Phthalazine **20c** showed a10 times MT_2-selectivity over MT_1

Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Dr. Philippe Delagrange a Project Director in Preclinical Drug Development, and Dr. Daniel Henri CAIGNARD a Chemistry Project Leader who are working for Les Laboratoires Servier, the marketer of the Valdoxan (Agomelatine), declare that their positions within Les Laboratoires Servier have no influence on the work reported in this manuscript.