◎ CASE HISTORY

Agomelatine, the first melatonergic antidepressant: discovery, characterization and development

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Abstract | Current management of major depression, a common and debilitating disorder with a high social and personal cost, is far from satisfactory. All available antidepressants act through monoaminergic mechanisms, so there is considerable interest in novel non-monoaminergic approaches for potentially improved treatment. One such strategy involves targeting melatonergic receptors, as melatonin has a key role in synchronizing circadian rhythms, which are known to be perturbed in depressed states. This article describes the discovery and development of agomelatine, which possesses both melatonergic agonist and complementary 5-hydroxytryptamine 2C ($5-HT_{2C}$) antagonist properties. Following comprehensive pharmacological evaluation and extensive clinical trials, agomelatine (Valdoxan/Thymanax; Servier) was granted marketing authorization in 2009 for the treatment of major depression in Europe, thereby becoming the first approved antidepressant to incorporate a non-monoaminergic mechanism of action.

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Depression is a complex, heterogeneous and incapacitating disorder that is associated with a heavy burden to patients and their families, and to society. Although currently available antidepressants have genuine value in the treatment of this disorder, they take several weeks to exert full efficacy, many patients respond inadequately, co-morbid symptoms are often not well controlled and all can present problems of poor tolerance[1–3]. Given this, increasing attention is being paid to cognitive and behavioural interventions. In addition, electroconvulsive therapy remains an option for refractory patients, deep-brain stimulation may eventually become of broader utility and the importance of preventative self-help in vulnerable subjects should not be underestimated[1,2,4,5]. Nonetheless, pharmacotherapy is likely to remain at the core of treatment for an established episode of major depression, so there is a clear need for new and improved antidepressants[1,2,6–8].

The first antidepressants — tricyclics (such as amitriptyline), which suppress the reuptake of mono-amines, and monoamine oxidase inhibitors (such as phenalzine), which interfere with their catabolism — were introduced in the 1960s and the 1970s. Their availability transformed the treatment of depression, but their limitations, in terms of side effects and safety, soon became apparent[1–3,7]. In their wake, the 1980s and the 1990s witnessed the introduction of more specific inhibitors of serotonin (also known as 5-hydroxytryptamine; 5-HT) and/or noradrenaline reuptake. Selective serotonin reuptake inhibitors (SSRIs), noradrenaline reuptake inhibitors (NARIs) and serotonin/noradrenaline reuptake inhibitors (SNRIs) possess improved safety margins as well as utility for treating co-morbid anxiety, but offer no real gain in efficacy over their first-generation counterparts[2,3,9,10]. Furthermore, they all, including mirtazapine, an atypical agent that does not modify serotonin or noradrenaline reuptake, act through monoaminergic mechanisms[2,11].

Since then, the development of new antidepressants can be characterized primarily as variations on a theme (although no two agents are identical so it is important to have several drugs per class)[1,2,6,7], rather than genuine mechanistic originality. Nonetheless, it would be naive to think that improved monoaminergic antidepressants could not be developed, as a palette of around 30 receptors awaits more sophisticated exploitation[2,11]. Furthermore, the association of a monoaminergic antidepressant with an additional mechanism, such as the co-administration of an SSRI with lithium for resistant depression, remains an attractive and clinically validated option[2,12,13].

Box 1 | **Melatonin, circadian rhythms and depression**

Major depression is frequently accompanied by alterations in circadian rhythms of behaviour, sleep, core temperature and the secretion of cortisol and other hormones[21–23]. Although changes are heterogeneous, frequent findings in patients with depression include a blunted amplitude of daily rhythms and poor responsiveness to environmental (photic and non-photic) entraining stimuli. Phase advances tend to predominate, but phase delays have also been described and they are typical of seasonal affective disorder[46,119]. Circadian disruption is linked to, and may partially be a consequence of, the changes in behaviour and sleep patterns that accompany depression; desynchronization may also be triggered by an intrinsic disorganization of the suprachiasmatic nucleus[2,127].

On the other hand, circadian disturbances may be provoked by an abnormal pineal output of melatonin, a key synchronizer of biological rhythms and sleep, of which the secretion is tightly coupled to light–dark and seasonal cycles[34,101] (FIG. 1). In line with this view, depression is associated with an altered diurnal rhythm of melatonin output, including a blunted night time surge[21–23,34,101,127]. Furthermore, although data are limited, melatonin production may be lower in depression, and an enhancement in circulating levels of melatonin has been correlated with effective treatment by certain antidepressants[2,21,127]. Administration of melatonin itself is ineffective in major depression[128]. However, it can improve sleep patterns, and 'chronotherapeutics' such as light and circadian behavioural therapy are also useful in this regard[46,101]. Taken together, the above observations support the notion that the re-coordination of biological rhythms by recruitment of melatonergic mechanisms is a therapeutically relevant strategy for improving depressed states.

Major depression
A serious disorder characterized by depressed mood (sadness) and anhedonia (inability to experience pleasure). Other important features include feelings of despair, worthlessness, suicidal ideation, lethargy or agitation, and insomnia (or hypersomnia). Co-morbid anxiety, cognitive impairment, sexual dysfunction and circadian desynchronization are common. For diagnosis, symptoms must be intense, disruptive and present more or less constantly for at least a fortnight. The lifetime risk of major depression is ~10%.

Tricyclics
Named after their chemical structure, tricyclic antidepressants inhibit the reuptake of serotonin and/or noradrenaline, activity of which are thought to be deficient in (at least some) patients with depression. Tricyclics can be highly effective, but their use is complicated by side effects due to other actions at, for example, central muscarinic receptors and cardiac ion channels.

Non-monoaminergic mechanisms have also been explored in the quest for improved antidepressants. Such research programmes have focused mainly on highly selective ligands of targets such as neurokinin 1 and corticotropin-releasing factor 1 receptors[14,15]. Unfortunately, clinical trials have been disappointing[16,17], perhaps, ironically, precisely because these drugs are so selective[2]. Pilot therapeutic trials have reported rapid antidepressant actions of NMDA (N-methyl-D-aspartate) receptor antagonists but, even for subunit-selective agents, reservations remain concerning psychomimetic side effects[18]. Agents designed to directly manipulate intracellular signals controlling neurogenesis and 'neuronal resilience' are also of interest, but pose questions of specificity and safety[2,7,19,20].

An alternative, appealing and innovative approach towards improved treatment of depression focuses on melatonin, an important regulator of circadian rhythms, which are perturbed in depression[21–23] (BOX 1). This Case History chronicles the discovery, characterization and development of the novel melatonergic antidepressant agomelatine, which represents two significant firsts: the first antidepressant designed to counter the perturbed biological rhythms linked to depressed states, and the first clinically available antidepressant to incorporate a non-monoaminergic mechanism of action.

From the pineal gland to agomelatine

The pineal gland, which produces melatonin, is a phylogenetically ancient structure. It has been discussed in scientific tracts since antiquity[24,25], but melatonin itself was discovered only about 100 years ago (in 1917) when McCord and Allen showed that extracts of the bovine pineal gland altered skin pigmentation in tadpoles. Forty years were to pass before the isolation and chemical identification of melatonin (N-acetyl-5-methoxytryptamine)

by Lerner in 1958 (REFS 24,26). Although high-affinity melatonin binding sites had been pharmacologically characterized in the bovine brain by 1979 (REF. 27), and were subsequently identified in the rat hypothalamus in the 1980s[28], it took another 40 years until the first melatonergic receptor was cloned from melanophores of *Xenopus laevis* in the 1990s[29].

The impetus for launching a research and development programme dedicated to melatonergic agonists at Servier Research Group goes back to 1988, when one of the authors (B.G.-L.) arrived following completion of a doctoral thesis on melatonin. Before the 1980s, the principal function of melatonin was thought to be in the control of reproduction[30,31]. However, it had become clear that melatonin behaves as a non-photic 'message' that interacts with photic signals in the control of circadian and diurnal cycles (FIG. 1). This was a particularly important observation as plasma concentrations of melatonin display marked circadian periodicity, with a peak during the nocturnal phase in both diurnal and nocturnal mammalian species. Overall, melatonin emerged to have a fundamental role in the synchronization of circadian rhythms[26,30] that are disorganized in central nervous system disorders such as depression[21–23,32] (BOX 1).

The notion of exploiting melatonergic ligands as therapeutic agents[33,34] was enthusiastically received and a series of naphthalene derivatives of melatonin were prepared[35,36]. These structures were intended to be both patentable and at least as potent as melatonin. Furthermore, inasmuch as the naphthalene ring of the derivatives is more lipophilic than the indole of melatonin, an additional objective was improved penetration into the brain. At that time, no cloned melatonergic receptors were available for study and procedures for performing binding studies in rodents had not been described. Consequently, using the radioligand [^{125}I]iodomelatonin, it was decided to examine the interaction of ligands with melatonin binding sites located in the posterior pituitary of sheep[28]. As illustrated in FIG. 2, melatonergic receptors couple via a $G\alpha_{i/o}$ protein to adenylyl cyclase. Hence, using an enzymatic assay, the functional actions of ligands were evaluated *in vitro* by determination of their influence on forskolin-stimulated cyclic AMP production in cells derived from ovine pars tuberalis[36]. Furthermore, in electrophysiological investigations, agonist properties of the derivatives were verified at the crucial population of melatonergic receptors localized in the suprachiasmatic nucleus (SCN). This work was performed in Syrian hamsters, a classical species used for the study of circadian rhythms[37,38].

Collectively, these studies showed that the naphthalene derivatives of melatonin behaved as high-affinity agonists. The compound known as S20098 — later named agomelatine — was identified as the most promising drug based on its overall profile[36,38,39]. Agomelatine potently binds to melatonin receptors, suppresses cAMP formation and mimics the actions of melatonin by dose-dependently inhibiting the firing rate of SCN neurons[38]. These observations were later substantiated when it was shown that agomelatine also potently activates cloned human melatonin 1 (MT$_1$) and MT$_2$ receptors[39] (FIG. 2).

Figure 1 | The relationship between melatonin, the suprachiasmatic nucleus and circadian rhythms: melatonergic actions of agomelatine *in vivo*. a | Light activates the glutamate (GLU)-containing retinohypothalamic tract (RHT) that runs from the eye to the suprachiasmatic nucleus (SCN). Through a polysynaptic projection, the SCN functionally inhibits the activity of the superior cervical ganglia (SCG), which supply the pineal gland with an excitatory, noradrenaline (NA)-containing input. This circuit allows light to suppress the production and release of melatonin from the pineal gland and, correspondingly, melatonin secretion is increased in the dark period. Melatonin reciprocally activates neurons in the SCN by actions at melatonin 1 (MT_1) and MT_2 receptors. Serotonergic input from the raphe nucleus modulates the SCN through actions at serotonin (also known as 5-hydroxytryptamine; 5-HT) receptor $5-HT_{2C}$ and other classes of 5-HT receptor. Daily behaviours likewise influence output from the SCN, the neuronal master clock for coordinating circadian rhythms. **b** | Melatonergic receptors recognized autoradiographically in the SCN using [^{125}I] iodomelatonin. **c** | Locomotor activity rhythms of rats drift backwards when the onset of the dark period is delayed by several hours. Daily administration of agomelatine (3.0 mg per kg, intraperitoneally) resynchronizes rhythms to their usual circadian pattern (dark period commencing at 18:00 hours)[45].

Monoamine oxidase inhibitors
These antidepressants mainly act by inhibiting the breakdown of serotonin and noradrenaline by monoamine oxidase A. They are clincially efficacious but, in particular for non-reversible inhibitors, blockade of the catabolism of dietary amines like tyramine can provoke a potentially dangerous hypertension.

Suprachiasmatic nucleus
(SCN). The bilateral SCN, which is located just above the optic tract at the base of the hypothalamus, acts as the master pacemaker for the body's circadian rhythms. Its neurons discharge rhythmically even when isolated. *In situ,* they are entrained to the daily light–dark cycle by information received via the retinohypothalamic pathway. Suprachiasmatic output influences the secretion of melatonin, which itself modulates the activity of the SCN.

Agomelatine
In 1997, following an application to the World Health Organization, S20098 was attributed the international non-proprietary name agomelatine in recognition of its innovative melatonergic profile, as compared with other antidepressants acting via monoaminergic mechanisms.

Phase advance and phase delay
Exposure to stimuli such as light and melatonin can shift circadian rhythms of the sleep–wake cycle and motor activity either forward (phase advance) or backwards (phase delay). For example, a brief pulse of light just after the onset of the dark period leads to a phase delay. Phase advances and phase delays in patients with depression are symptomatic of circadian disorganization and probably reflect a dysfunction of the suprachiasmatic nucleus.
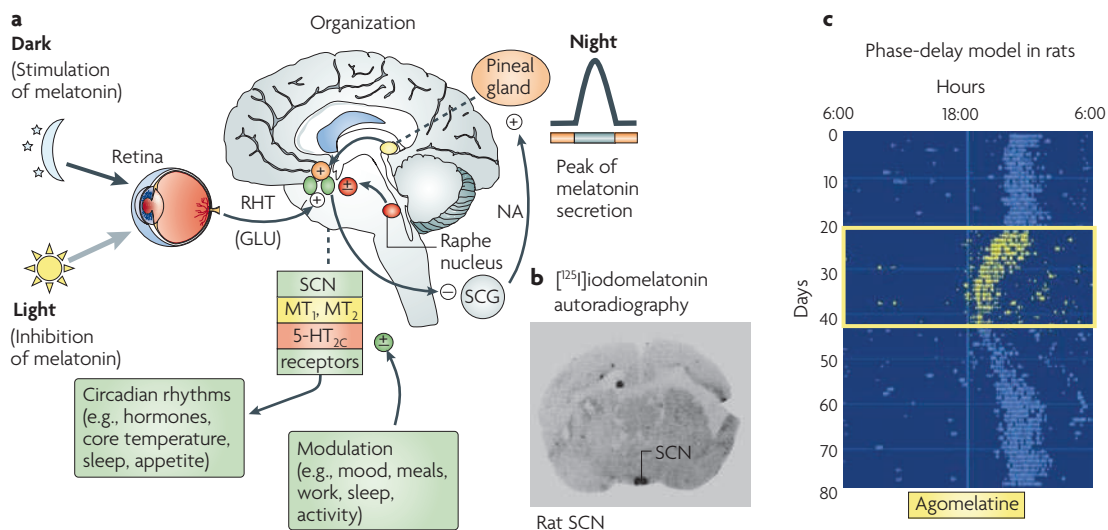
Studies[40–43] relevant to another interesting issue — how agomelatine affects the responsiveness of MT_1 and MT_2 receptors — are discussed later.

The bioavailability of agomelatine was discovered to be modest. Nevertheless, it displayed robust *in vivo* activity both in animals and in humans, as described in the next section.

Resynchronizing properties of agomelatine
A major goal of the *in vivo* studies of agomelatine was to show that it normalizes disturbances of circadian rhythms, especially in models related to depressed states. In rats maintained in total darkness, the normal time for onset of locomotor activity drifted backwards and chronic administration of agomelatine reversed this shift, an effect well documented for melatonin[44]. It was subsequently discovered that agomelatine shows resynchronizing actions in several other conditions. For instance, it restored circadian rhythms in a model of jet lag that involved a phase advance of the light–dark cycle[45]. It also resynchronized circadian rhythms in a paradigm involving a phase delay, an observation pertinent both to clinically defined delayed sleep disorder syndrome and to the disruption of circadian rhythms seen in seasonal depression[46,47] (BOX 1; FIG. 1). Interestingly, it was recently reported that the intensity of symptoms in major depression is correlated with circadian misalignment; that is, the more delayed the pacemaker relative to the timing of sleep, the more severe the depressed state[48].

Ageing is also associated with a weakened responsiveness of the circadian clock to environmental stimuli. Agomelatine reinstated both circadian rhythms of wheel-running activity in aged hamsters[49], as well as diurnal rhythms of motor activity and core temperature in aged (about 2 years old) rats[50]. These findings are relevant to depression as the blunted amplitude of diurnal rhythms in aged rats resembles the alterations seen in certain patients with depression[21–23] (BOX 1). Finally, agomelatine reinstated normal circadian rhythms in trypanosome-infected rats displaying a disrupted sleep–wake cycle[51]. These studies were all performed in nocturnal animals (rodents and hamsters), so the resynchronizing actions of agomelatine were later corroborated in the African rat, *Arvicanthis mordax*, a diurnal species like humans[52].

Collectively, the above observations prompted a study in healthy volunteers in which agomelatine phase-advanced rhythms of body temperature without modifying circulating levels of melatonin[53]. This observation coincided with experiments showing that pinealectomy does not modify the influence of agomelatine on circadian rhythms in rodents[54,55]. Independence from the pineal gland suggested that agomelatine was acting, as suspected, in the SCN. However, melatonergic receptors are also present in other cerebral regions[30,56] (see below), so the finding that lesions of the SCN abolished the resynchronizing activity of agomelatine was an important one[54].
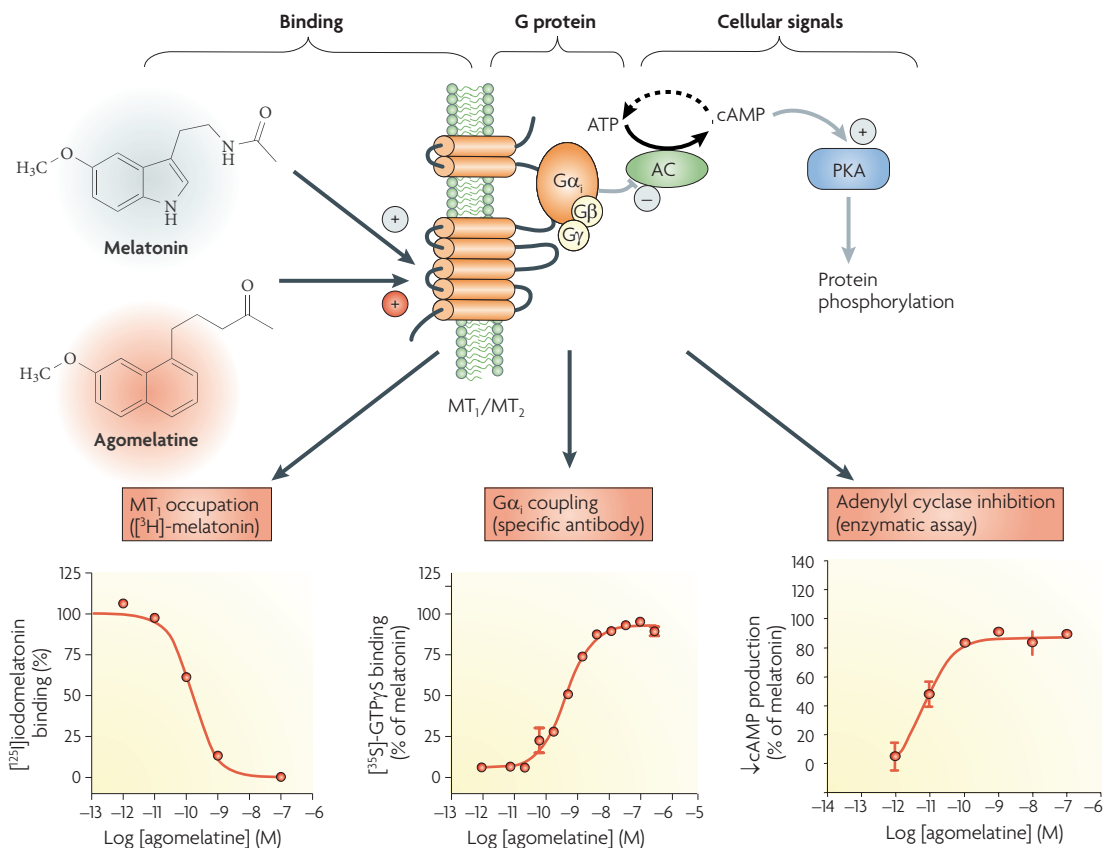
Figure 2 | **Agonist properties of agomelatine at melatonergic receptors coupled via Gα$_i$ to inhibit adenylyl cyclase.** Both melatonin receptors (MT$_1$ and MT$_2$) couple via Gα$_i$ to inhibit adenylyl cyclase (AC). This leads to reduced formation of cyclic AMP (cAMP) from ATP and hence decreased activity of protein kinase A (PKA), which phosphorylates a variety of cellular substrates. Melatonin is the endogenous ligand of MT$_1$ and MT$_2$ receptors and its actions are mimicked by the naphthalene analogue agomelatine. This is illustrated for MT$_1$ receptors in the lower panels, which show displacement of the MT$_1$ radioligand [^{125}I]iodomelatonin by agomelatine, enhancement of [^{35}S]-GTPγS binding to Gα$_i$ and suppression of cAMP production (similar data are seen with MT$_2$ sites[41]).

Unedited 5-HT$_{2C}$ receptors
5-HT$_{2C}$ receptors in humans and other species are present in 20 or more isoforms, reflecting a contrasting (three) amino acid sequence located in the second intracellular loop, which is involved in signal transduction. Alterations in this sequence are caused by post-translational (adenosine to inosine) editing of mRNA. Unedited (INI) sites are constitutively active, whereas highly edited sites (like VSV) are not.

Constitutive activity
Some G protein-coupled receptors are active even in the absence of agonists. This reflects the spontaneous interaction of the receptor with G proteins and other transduction mechanisms, and is usually reflected in a resting level of agonist-independent signal transduction and/or receptor endocytosis into the interior of the cell.

Inverse agonist
Inverse agonists suppress the resting (constitutive) activity of G protein-coupled receptors in the absence of agonists.

Neutral antagonist
Neutral antagonists alone do not affect basal activity. Instead, they normalize signalling by blocking the actions of both agonists and of inverse agonists, thereby returning activity to baseline values.

A major circadian effect of agomelatine turned out to be a phase advance, which was expressed most markedly at the moment of the light–dark transition. That is, its maximal effectiveness coincided with the onset of the elevation in night time melatonin secretion and with the time of maximal melatonin receptor sensitivity[25,26,30,37]. Furthermore, short-term (1 hour) exposure to agomelatine was sufficient for expression of its resynchronizing activity in humans, which was even seen the following day[53,57,58]. This was a significant finding as the half-life of agomelatine in humans is around 2 hours. Subsequent work confirmed that transient activation of melatonergic receptors at the crucial time point is sufficient for enduring effects. This could be due to the induction of kinase-mediated cascades of protein phosphorylation, to alterations in the release of neurotransmitters that are only slowly cleared from the synaptic cleft and to long-term modifications of synaptic plasticity in the SCN and elsewhere[19,54,55].

Interaction with 5-HT$_{2C}$ receptors
In the late 1990s, it was decided to investigate the potential effects of agomelatine at 5-HT$_{2C}$ receptors, an interaction that had just been detected in a standardized binding screen. Interestingly, post-transcriptional modification of 5-HT$_{2C}$ receptors by mRNA editing had just been discovered to generate structurally distinct isoforms[59–61]. Unedited 5-HT$_{2C}$ receptors have constitutive activity, meaning that inverse agonists decrease baseline signalling and encourage the migration of 5-HT$_{2C}$ receptors from the cytoplasm to the plasma membrane[61,62]. Conversely, neutral antagonists have no activity alone, yet block the actions of agonists and inverse agonists. A further intriguing feature of 5-HT$_{2C}$ sites was their coupling to several cellular signals that could be differentially influenced by various ligands, a phenomenon termed ligand-biased signalling[60,63]. Nonetheless, the canonical signalling pathway of 5-HT$_{2C}$ receptors was well established to be Gαq-mediated activation of phospholipase C (FIG. 3). Thus, after confirming that agomelatine displaces the radiolabelled antagonist, [^{3}H]mesulergine, from recombinant human 5-HT$_{2C}$ receptors, it was shown to competitively antagonize the activation of Gαq and phospholipase C by serotonin[64] (FIG. 3). These observations were extended to a further G protein, Gα$_i$, suggesting that agomelatine acted as a broad-based antagonist at 5-HT$_{2C}$ receptors coupled to
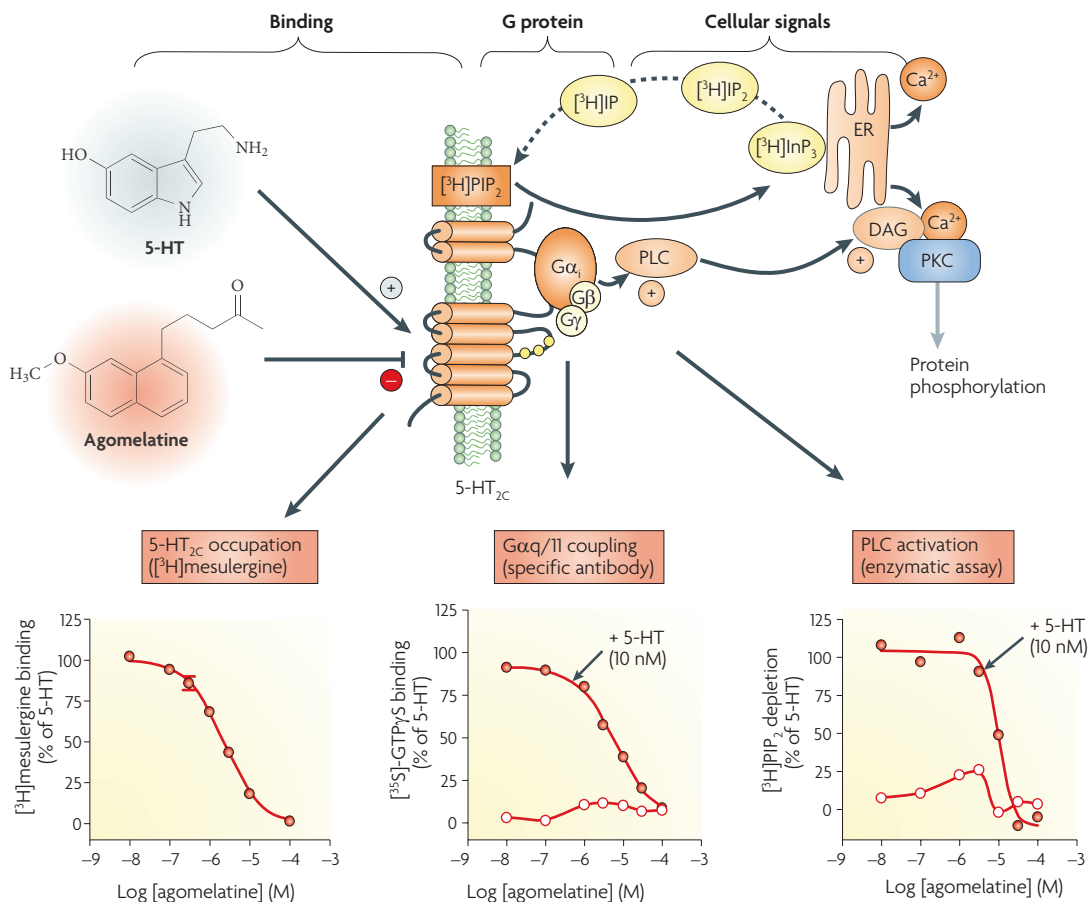
Figure 3 | Antagonist properties of agomelatine at 5-HT$_{2C}$ receptors coupled via Gαq to activation of phospholipase C. 5-hydroxytryptamine 2C (5-HT$_{2C}$) receptors couple via Gαq/11 to activate phospholipase C (PLC), which generates diaminoglycerol (DAG) and inositol-1,4,5-triphosphate (InsP$_3$) from membrane-localized phosphoinositides (phosphatidylinositol 4,5-bisphosphate; PIP$_2$). InsP$_3$ stimulates the release of calcium from the endoplasmic reticulum (ER), which, together with DAG, activates protein kinase C (PKC), leading to the phosphorylation of various cellular substrates. The actions of 5-HT at 5-HT$_{2C}$ receptors are blocked by the neutral antagonist agomelatine (inactive alone)[64]. Illustrated in the lower panels is the displacement of the 5-HT$_{2C}$ radioligand [^{3}H]mesulergine by agomelatine, blockade of 5-HT-elicited [^{35}S]-GTPγS binding to Gαq/11 and antagonism of 5-HT-induced depletion of [^{3}H]PIP$_2$. IP, inositol phosphate; IP$_2$, inositol 1,4-bisphosphate.

diverse signalling pathways (FIG. 3). Moreover, agomelatine normalized signalling at 5-HT$_{2C}$ sites, rather than suppressing it below basal levels, which is consistent with neutral antagonist properties[60,64].

The observation that agomelatine blocked 5-HT$_{2C}$ receptors was of considerable interest as they fulfil major roles in the control of mood and the response to stress[65,66] (BOX 2). Furthermore, serotonin synthesis is highly circadian, the SCN is intensely innervated by serotonergic pathways emanating from the raphe nucleus, and SCN-localized 5-HT$_{2C}$ receptors contribute to the integration of photic and non-photic modulation of circadian rhythms[67–70] (FIG. 1). Owing to species differences, the precise role of 5-HT$_{2C}$ receptors as modulators of SCN activity in humans remains uncertain[70,71]. Nevertheless, actions of agomelatine at melatonergic and 5-HT$_{2C}$ receptors co-localized in the SCN may participate in its influence on circadian rhythms and in its resynchronizing actions in depression.

Actions at melatonergic and 5-HT$_{2C}$ receptors

The discovery of 5-HT$_{2C}$ antagonist properties for agomelatine was clearly important. However, it was necessary to understand the issue of the disparity between its high affinity for human MT$_1$ and human MT$_2$ sites and its substantially (more than 100-fold) lower affinity at human 5-HT$_{2C}$ receptors. Although it is hard to compare potencies for agonism at one site with antagonism at another, the question of whether the affinity of agomelatine for 5-HT$_{2C}$ receptors is biologically meaningful had to be asked. The best way to address this question was through *in vivo* experiments using several behavioural and neurochemical procedures. It was shown that agomelatine, but not melatonin, blocked cerebral populations of 5-HT$_{2C}$ receptors in rodents[64]. These effects of agomelatine were seen at doses (2.5–40.0 mg per kg, intraperitoneally) yielding low micromolar levels in the brain, comparable to its affinity for 5-HT$_{2C}$ receptors. Moreover, in comparison with other

Box 2 | **5-HT$_{2C}$ receptors, mood and depression**

5-hydroxytryptamine$_{2C}$ (5-HT$_{2C}$) receptors are present in the suprachiasmatic nucleus (SCN), where they modify the response of intrinsic neurons to photic input[67,69,70]. Interestingly, a polysynaptic circuit runs from the SCN to the ventrotegmental nucleus, the origin of mesocortical and mesolimbic dopaminergic pathways[129]. This neural link provides an anatomical substrate for an indirect influence of SCN-localized 5-HT$_{2C}$ receptors on ascending dopaminergic transmission. Furthermore, 5-HT$_{2C}$ receptors are enriched in the ventrotegmental area itself and in the locus coeruleus, the source of forebrain adrenergic pathways[2,77]. In these nuclei, as in the frontal cortex, excitatory 5-HT$_{2C}$ receptors are localized on GABA (γ-aminobutyric acid)-ergic interneurons. Hence, their blockade disinhibits frontocortical dopaminergic and adrenergic transmission (FIG. 5), the activity of which may be compromised in depression[2,61]. 5-HT$_{2C}$ receptors are also concentrated in limbic structures such as the frontal cortex, the amygdala, the hippocampus and the septum, which have major roles in the control of mood and in the aetiology of anxio-depressive states[65,66].

Activity at 5-HT$_{2C}$ receptors seems to be enhanced in depression, whereas a decrease is elicited by long-term administration of certain antidepressants, as well as sleep deprivation and electroconvulsive therapy that similarly alleviate depressed mood[2,4,65,66]. Antidepressants such as the tricyclic clomipramine, and the atypical agent mirtazapine, antagonize 5-HT$_{2C}$ receptors[61,62], and small-scale investigations of mixed 5-HT$_{2C}$/5-HT$_{2A}$ antagonists such as ritanserin suggested that mood may be improved[66]. Furthermore, selective 5-HT$_{2C}$ receptor antagonists display antidepressant and anxiolytic properties in rodents, and both reduced sensitivity to stress and an anxiolytic phenotype have been reported in mice genetically deprived of 5-HT$_{2C}$ receptors[65,66,82,123]. Finally, 5-HT$_{2C}$ receptor antagonists promote slow-wave sleep[89,91] and libido[2,110]. Collectively, these observations suggest that 5-HT$_{2C}$ receptor antagonism should favourably influence mood, circadian synchronization and sleep quality, while preserving sexual function.

Forced swim test
In this test of potential antidepressant properties, rodents are placed for 15 minutes in a cylinder of water (room temperature) from which they cannot escape. The following day, in the course of a second session, the time of immobility is measured as an index of despair. Given either chronically or acutely (on the test day), antidepressants reduce immobility time.

Chronic mild stress
A procedure whereby rodents are exposed for a period of about 5 weeks to minor daily stressors like wetting the sawdust, noise, moving the cage and so on. This leads to a progressive state of anhedonia (inability to experience reward), reflected in a reduction in the preference of sucrose over water. This state can be reversed by chronic administration of antidepressants.

selective antagonists, the potency of agomelatine *in vivo* correlated well with its affinity for 5-HT$_{2C}$ receptors *in vitro*[66].

In fact, there is nothing unusual about such modest potency for a drug. For example, the SNRI venlafaxine possesses only micromolar affinity for noradrenaline transporters, yet exerts adrenergic actions in rats and humans[2,11,72]. Encouragingly, the difference between the doses at which substantial 5-HT$_{2C}$ antagonist and mela-tonergic agonist actions of agomelatine (shown at doses 1.0–3.0 mg per kg, intraperitoneally) were observed was far less pronounced *in vivo* than *in vitro*. Possibly, a very high degree of occupation of melatonergic sites is needed for their robust activation[56]. This issue remains to be further evaluated.

Irrespective of the explanation, at the dose range over which agomelatine possesses antidepressant properties in rodents, both melatonergic and 5-HT$_{2C}$ receptors should be activated and blocked, respectively, and this probably also applies for humans[73] (FIG. 4). Recent magnetic resonance imaging work has confirmed that agomelatine blocks 5-HT$_{2C}$ sites in the rat brain[74] and a similar study is planned in humans.

Antidepressant profile of agomelatine

Actions in diverse experimental models. In experimental studies designed to explore the antidepressant potential of agomelatine, three complementary strategies were adopted (TABLE 1). First, its actions were evaluated in several well-established procedures reflecting core clinical features of depression. Second, potential resynchronizing properties were examined in depression models characterized by disrupted circadian rhythms. Third, the influence of agomelatine on neurobiological parameters known to be dysfunctional in depression was explored: frontocortical dopaminergic and adrenergic transmission (compromised); hippocampal neurogenesis (suppressed), and activity of the hypothalamic–pituitary–adrenal (HPA) axis (overactivated)[2,7,20].

One major issue with evident clinical repercussions was when to administer agomelatine. At least for its melatonergic properties, this was likely to make a real difference and, as a general principle, it is important not only to give the right medicine, but also to give it at the right time. Consistent with our original supposition (see above), evening administration seemed preferable since this corresponds to the onset of night time melatonin secretion for both diurnal and nocturnal species[26,31,33] (FIG. 1). Moreover, the resynchronizing effects of ago-melatine had been observed when taken in the evening. Thus, it was decided to continue with this basic schedule, with the exception of some acute studies.

Early results showing that agomelatine had activity in the forced-swim test and the chronic mild stress procedures were vital for initiation of the clinical programme in depression[75,76] (TABLE 1). Furthermore, enhanced dopamine and noradrenaline release in the frontal cortex provided a compelling basis for therapeutic efficacy[64] (BOX 2; FIG. 5). Interestingly, in comparison to many other antidepressants, agomelatine selectively reinforced frontocortical versus subcortical dopamin-ergic transmission[2,77]. Subsequently, it was discovered that agomelatine shares two other interrelated actions of antidepressants that were beginning to attract considerable attention: it promoted neurogenesis and it enhanced levels of brain-derived neurotrophic factor in the hippocampus[19,20,78,79].

Synergistic roles for melatonin agonism and 5-HT$_{2C}$ antagonism. The breadth of evidence supporting the antidepressant properties of agomelatine (TABLE 1), together with the resynchronizing actions outlined above, provided a convincing rationale to initiate clinical trials. However, the question of how agomelatine was exerting its antidepressant actions remained. Could its effects be attributed to its melatonergic agonism, to its 5-HT$_{2C}$ receptor antagonism and/or to a combination of these effects? It was unlikely that there was going to be a unitary answer, so the actions of agomelatine were compared to those of both melatonin and 5-HT$_{2C}$ antagonists. Furthermore, in certain studies, mechanistic insights were afforded by the use of melatonergic antagonists.

The possibility that melatonergic mechanisms participate in the antidepressant actions of agomelatine is supported by the presence of MT$_1$ and/or MT$_2$ receptors in human hippocampus, nucleus accumbens and frontal cortex, where they are thought to have a role in the control of mood[56]. Underscoring the possible participation of melatonergic receptors in the antidepressant properties of agomelatine, melatonin mimicked its ability to counter circadian disruption in transgenic mice expressing a mutant glucocorticoid gene, which leads to disinhibition of the HPA axis[80]. On the other hand, 5-HT$_{2C}$ receptor antagonists mimicked the reinforcement by agomelatine
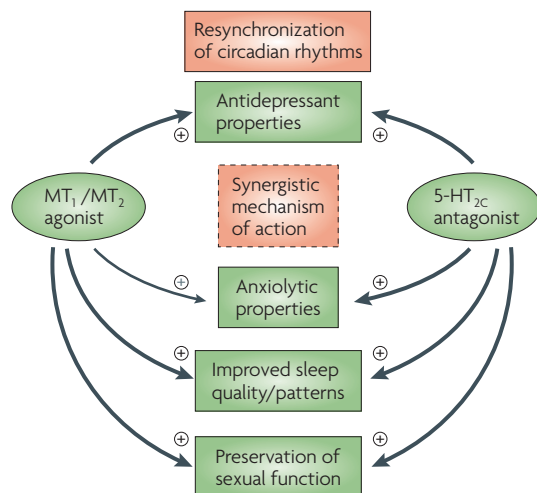
Figure 4 | **Overview of the mechanism of antidepressant action of agomelatine.** Agomelatine possesses both melatonin receptor (MT_1 and MT_2) agonist and 5-hydroxytryptamine receptor ($5-HT_{2C}$) antagonist properties. These properties act in a complementary and perhaps synergistic manner to improve depressed states by resynchronization of circadian rhythms, enhancement of dopaminergic and adrenergic input to the frontal cortex, induction of neurogenesis, as well as through other mechanisms (TABLE 1). Furthermore, melatonergic agonism and $5-HT_{2C}$ receptor antagonism also act in harmony to favourably influence anxious symptoms, sleep and sexual function.

Learned helplessness
This refers to the observation that exposure to uncontrollable stress can compromise the ability to learn to escape from a subsequent aversive situation. In the learned helplessness procedure, rats are exposed to a sequence of inescapable foot-shocks in a chamber and then evaluated in an avoidance-conditioning test (escape from acute shock) in a two-compartment box. The number of escape failures is considered an index of helplessness. Antidepressants given before the test reinstate escape-directed behaviour.

Tree shrew
(Also known as Scandentia) Tree shrews are united with primates in the super-order Euarchonta, itself fused with rodents and lagomorphs in the Euarchontoglires. They are diurnal, territorial animals that live in family-based social groups. Contact of a defeated subordinate with a dominant male provokes marked stress-related changes in behaviour, endocrine secretion (hypothalamic–pituitary–adrenal axis overactivity) and physiology, as well as disruption of circadian rhythms.

of frontocortical dopaminergic and adrenergic input, and its reduction of hyperactivity in rats subjected to olfactory bulbectomy, a model of depression-related agitation[64,66,81,82]. Intriguingly, however, although a melatonin antagonist abrogated the effect of agomelatine in the learned helplessness procedure, its actions were not reproduced by melatonin or by $5-HT_{2C}$ receptor antagonists[83]. It could therefore be inferred that the combined actions of agomelatine at melatonergic and $5-HT_{2C}$ sites were required for efficacy. Consistent with this notion, although $5-HT_{2C}$ antagonists mimic the induction by agomelatine of cellular proliferation in the hippocampus, they do not reproduce its enhancement of cellular survival or of levels of brain-derived neurotrophic factor, which are only slightly increased by melatonin[79].

In a further model with a prominent circadian element, psychosocial stress in the tree shrew, *Tupaia belangeri*, agomelatine opposed the disruption of diurnal rhythms of cortisol secretion and core temperature. Again, melatonin was inactive, whereas a $5-HT_{2C}$ antagonist merely blunted hypercortisolaemia[84,85]. An elegant illustration of the roles of both melatonin and $5-HT_{2C}$ receptors was acquired in the above mentioned study of chronic mild stress. Agomelatine was active on administration both in the evening (mirrored by melatonin) and in the morning (mimicked by a $5-HT_{2C}$ antagonist)[75]. By contrast, and dependent on experimental conditions, both melatonin and $5-HT_{2C}$ antagonists are active in the forced-swim test[66,82,86,87].

Collectively, the above results support the assertion that neither melatonin agonism nor $5-HT_{2C}$ receptor antagonism can fully account for the antidepressant properties of agomelatine. Instead, dual actions at both melatonin and $5-HT_{2C}$ receptors underpin its broad-based antidepressant profile in animal models, which was progressively elucidated in parallel with the clinical studies described below[73,88].

Transition to the clinic
Early Phase I trials showed that agomelatine (5–1,200 mg) was well tolerated, and 800 mg was defined as the maximal, well-tolerated dose based on one subject who experienced postural dizziness at 1,200 mg. Even at high doses, agomelatine was not associated with pronounced adverse effects; the most common adverse effects were mild sedation and headache[73,88]. This was encouraging, considering the problems of tolerability associated with other antidepressants[1–3].

Moreover, the good tolerability of agomelatine represents a reassuring safety cushion in the event of high exposure in certain patients. This was considered possible owing to inter-individual variability in the metabolism of agomelatine by the hepatic cytochrome P450 1A2, its major enzyme for degradation[73,88]. As anticipated from *in silico* metabolic predictions, *in vitro* studies of human microsomes and hepatocytes, and from *in vivo* work in animals, absorption of agomelatine was rapid and complete. However, bioavailability following oral administration was limited owing to a pronounced hepatic first-pass effect. Furthermore, as mentioned above, elimination was swift, with a half-life of about 2 hours.

Nonetheless, several reasons strongly suggested that this profile was compatible with therapeutic activity in patients with depression. First, preclinical work had shown robust activity of agomelatine in animals, including models of resynchronization and antidepressant properties. Second, as pointed out above, it should not be necessary to achieve full and 24 hour occupation of target receptors for clinical actions, and a half-life of 2 hours corresponded well to the dark-onset peak of melatonin release. Third, studies in volunteers given doses of 5 mg to 100 mg showed persistent phase advances of circadian rhythms, apparent even the day after treatment[57,58]. Finally, at similar doses, agomelatine slightly decreased core body temperature and elicited mild sedation[57,58], as would be expected on melatonin receptor stimulation[31].

Selection of doses for exploration of efficacy
A further important issue was the choice of dosage. Paradoxically, the good tolerance of agomelatine complicated this question, as maximal testable drug doses are often capped by unwanted side effects. The transition from Phase I to Phase II/III efficacy studies could not then be guided using now familiar tools such as pharmacological magnetic resonance imaging. In addition, efforts to develop positron emission tomography ligands for quantification of melatonin and $5-HT_{2C}$ receptor occupancy in humans had been, and remain, unsuccessful. In fact, the selection of 1 mg, 5 mg and
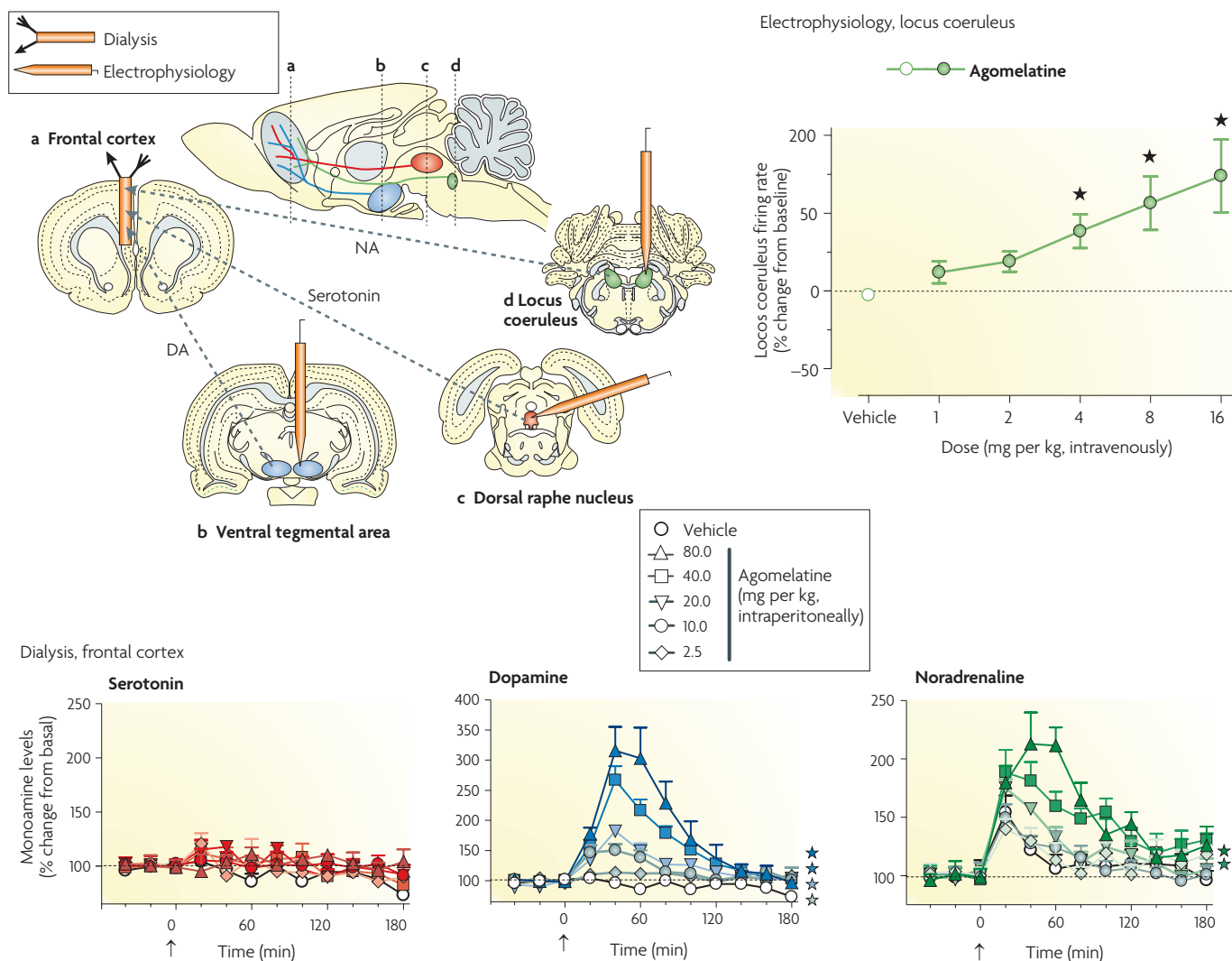
Figure 5 | Reinforcement of corticolimbic adrenergic and dopaminergic transmission by agomelatine.
The frontal cortex is innervated by ascending adrenergic, dopaminergic and serotonergic pathways originating in the locus coeruleus, the ventrotegmental area and the dorsal raphe nucleus, respectively. The influence of agomelatine on the activity of monoaminergic tracts has been measured in freely moving rats using dialysis coupled to high performance liquid chromatography and electrochemical detection to quantify extracellular levels of monoamines. In addition, the influence of agomelatine on the electrical activity of monoaminergic cell bodies has been evaluated in anaesthetized rats, as depicted here for the locus coeruleus (top right). Adrenergic and dopaminergic pathways are subject to indirect inhibition by 5-hydroxytryptamine 2C (5-HT_{2C}) receptors, which act through the excitation of GABA (γ-aminobutyric acid)-ergic interneurons. Accordingly, agomelatine dose-dependently increases the release of dopamine (DA) and noradrenaline (NA), but not serotonin in the frontal cortex. In parallel, it increases the firing rate of adrenergic neurons in the locus coeruleus[64]. Arrows represent injection of agomelatine. Stars represent $P < 0.01$ to vehicle (Dunnett's test after ANOVA).

25 mg for an initial dose-ranging trial was mainly guided by the above mentioned studies of phase-shifting in volunteers[57,58]. With hindsight, electroencephalography may have been instructive as we now know that both in rats[89] and in patients[90], agomelatine enhances restorative slow-wave sleep, an effect characteristic of 5-HT_{2C} receptor antagonists[66,89]. It should be noted that, in contrast to antidepressants suppressing monoamine reuptake[2,91], agomelatine does not reduce rapid eye movement sleep either in patients with depression[90] or in volunteers[92].

Thus, despite the current predilection for imaging, this is a good example of how a traditional pharmacodynamic approach could have been, and still can be, useful in estimating centrally active doses of a new drug.

Indeed, it did prove possible to establish doses of agomelatine that were effective in treating major depression. The following section summarizes the progression of efficacy and safety studies undertaken in almost 5,000 patients, primarily in Europe, but also in South Africa, South America, Australia and North America.

Table 1 | **Overview of the actions of agomelatine in experimental models relevant to depression**

Characteristic	Model	Species	Major observation	Refs
Cardinal symptom	Forced swim test (despair)	Rat	Decrease in immobility time	76
	Learned helplessness (resignation)	Rat	Disinhibition of suppressed responses	83
	Chronic mild stress (anhedonia)	Rat	Restored sucrose consumption	75
	Olfactory bulbectomy (motor agitation)	Rat	Decrease in hyperactivity	81
Circadian disruption	Mutated glucocorticoid receptor	Mouse	Decrease in perturbation of rhythms of corticosterone secretion	80
	Psychosocial stress	Tree shrew	Decrease in perturbation of rhythms of corticosterone secretion and core temperature	84,85
Biological substrate	Noradrenaline/dopamine in frontal cortex	Rat	Increase in extracellular levels	64
	Hippocampal neurogenesis	Rat	Increase in cellular proliferation and survival	78,79
	Levels of brain-derived neurotrophic factor	Rat	Increase in mRNA levels	79

Clinical evaluation for treating major depression

The clinical evaluation of agomelatine in major depression was designed around the requirements of the European Medicines Agency (EMA): first, demonstration of efficacy compared with placebo; second, maintenance of long-term effectiveness (prevention of relapse); third, comparisons to other clinically used antidepressants; and fourth, demonstration of a favourable risk–benefit profile. The results of all clinical trials, of which 3 out of 6 were positive, were submitted to the EMA and are publicly available online (see further information for the link to the assessment report for agomelatine). In addition, in a recent publication, the complete data set from both positive and negative trials is analysed in detail[93]. The following discussion focuses on the core clinical observations that drove further development, and which ultimately led to the recommendation of marketing authorization by the EMA under the trade names Valdoxan and Thymanax.

Short-term efficacy compared with placebo. A double-blind, paroxetine (SSRI)-validated, dose-ranging investigation was performed over 8 weeks at doses of 1 mg, 5 mg and 25 mg. It transpired that the 25 mg dose was the most effective based on the primary outcome criterion, the 17 item Hamilton depression rating scale (HAM–D)–total score (HAM–T)[94]. This conclusion was underpinned by the secondary outcome criteria: the percentage of patients that exhibited a greater than 50% reduction of HAM–T from baseline, and the clinical global impression severity (CGI–S) scores. Interestingly, as quantified by the Hamilton anxiety rating scale, agomelatine also alleviated anxiety. In addition, in corroboration with Phase I findings, tolerance was good.

The successful completion of this study triggered several other short-term Phase II efficacy trials against placebo. Unfortunately, an excessively high response rate

of 47–58% in the placebo arm negatively affected these investigations. As discussed elsewhere, this complex problem has compromised many trials of antidepressants and reflects multiple factors; notably, that patients were 'insufficiently' ill at inclusion, and that placebo is tantamount to a form of psychotherapy not encountered in the 'real world'[5,95,96]. To minimize the risk of an untoward placebo response, several methodological innovations were introduced. Only patients with moderate to severe depression were enrolled by imposing strict entry criteria at baseline. Furthermore, a disability scale of social and occupational dysfunction was adopted. These measures proved effective as two key Phase III studies using variable doses of agomelatine (25–50 mg) unambiguously substantiated its efficacy compared with placebo (response rates now approximately 35%)[73,88,97].

Demonstration of efficacy compared with other antidepressants. Several other head-to-head Phase III investigations were launched to compare agomelatine (25–50 mg) with the SNRI venlafaxine (75–150 mg) and with the SSRI sertraline (50–100 mg). Agomelatine showed efficacy at least comparable to these established drugs. In fact, it proved superior both to venlafaxine and to sertraline as judged by the HAM–D and CGI (improvement) secondary end points, respectively[98–100]. Notably, agomelatine was active across the whole patient sample and in patients with more severe depression (HAM–D ≥25; CGI–S ≥5 at baseline). In addition, the proportion of patients completing the 6-month treatment period was significantly higher for agomelatine than for the comparators[99,100] (FIG. 6b).

In view of the delay to full efficacy of other antidepressants[1,2,7], there was particular interest in the rate of onset, which had appeared more rapidly (week 2) in the first placebo-controlled study[94]. Furthermore, the

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Hamilton depression rating scale
(HAM-D). This scale is used to assess depressive states in patients. It incorporates various parameters, like depressed mood, feelings of guilt, insomnia and so forth. Severity is estimated numerically from zero (essentially normal); the higher the score, the more serious the depressed states.

observation that the proportion of responders at week 2 was greater in patients on agomelatine than those on sertraline was intriguing[99] as, in another study, the benefits of agomelatine translated into a greater early perception of well-being compared with venlafaxine at week 1 (REF. 98). The investigation evaluating agomelatine onset with venlafaxine highlighted two possible contributions to early symptomatic improvement: better daily functioning and improved sleep, which is often perturbed by SSRIs and SNRIs[2,3,91]. Thus, using the Leeds sleep evaluation questionnaire, the advantage of agomelatine relative to venlafaxine was apparent for both getting to sleep and quality of sleep by week 1, and persisted for 6 weeks[98]. These observations were consistent with findings pointing to early improvement in subjective sleep and daytime functioning for agomelatine compared with sertraline[99]. Both melatonin agonism and 5-HT$_{2C}$ antagonism probably participate in the ability of agomelatine to promote sleep[66,89,91,101]. Further study to examine the rate of onset of antidepressant actions of agomelatine is warranted.

Demonstration of relapse prevention compared with placebo. An initial, placebo-controlled investigation of relapse prevention was unsuccessful, largely due to a low rate of relapse in the placebo arm of the study. Nevertheless, this trial proved instructive in demonstrating that agomelatine was superior to placebo in patients severely ill at baseline, an observation coinciding well with the findings of the short-term trials described above[88,97]. The follow-up relapse prevention study was modified to resolve concerns that arose in the course of the first investigation. For example, a flexible dose regimen was exploited, permitting the (blinded) increase in dose from 25 mg to 50 mg at week 2. Furthermore, after 8–10 weeks of open treatment, patients entering the randomized, 6 months, double-blind continuation phase had to present HAM–D and CGI–S scores of ≤10 and ≤2, respectively, blinded both to the patient and the investigator. As exemplified by the primary outcome criterion (HAM–D), this study showed a twofold lower relapse rate during continuation in patients treated with agomelatine compared with patients treated with placebo[102] (FIG. 6a). Furthermore, a controlled extension of the study revealed that this advantage was sustained for up to 10 months, both in the entire population enrolled and in patients with more severe depression[103].

Lack of a discontinuation syndrome. In the studies of agomelatine described above, the lack of early relapse on switching to placebo argued for a minimal discontinuation syndrome. This attribute was underscored by a placebo-controlled study using paroxetine (20 mg) as an active comparator[104]. The comparative absence of an agomelatine discontinuation syndrome is advantageous as withdrawal can be a major problem with certain SSRIs, and discourages their use by patients[105]. It is difficult to extrapolate from the clinic back to the cell; nonetheless, long-term administration of 5-HT$_{2C}$ receptor inverse agonists increases the cell surface density and sensitivity of 5-HT$_{2C}$ sites, whereas the neutral antagonist, agomelatine, does not[61,62,64]. Consequently, stopping treatment

with agomelatine should not lead to deleterious effects due to activation by serotonin of re-exposed and hyperresponsive 5-HT$_{2C}$ receptors. However, this notion awaits formal proof.

Good tolerability compared with other antidepressants. One consistent finding throughout clinical testing of agomelatine has been its good tolerability, a potentially key benefit for initiation of treatment and long-term adherence[1,3]. Evidence for a favourable side-effect profile was apparent in short-term studies in which emergent adverse effects were almost indistinguishable from placebo[88,94,97]. Thus, based on the complete database of double-blind, 6 month studies, the only emerging adverse effect significantly associated with agomelatine (1,120 patients) compared with placebo (998 patients) was dizziness: 5.9% compared with 3.5%, respectively ($P<0.01$). Notably, in studies in which agomelatine was directly compared with venlafaxine and sertraline, the favourable safety profile of agomelatine translated into significantly fewer study dropouts due to side effects[98–100] (FIG. 6b).

Poor gastrointestinal tolerability and weight gain can sometimes provoke early cessation of treatment with other antidepressants[3,106]. By contrast, the gastrointestinal tolerability of agomelatine was good and it was weight-neutral. A lack of weight gain was especially reassuring as gene knockout studies in transgenic mice had suggested that obesity might be a drawback for the therapeutic use of 5-HT$_{2C}$ antagonists[65,66].

Even at supra-therapeutic doses, no clinically relevant changes were detected in biochemical, cardiac or cardiovascular parameters. Some isolated and reversible increases in serum alanine and/or aspartate transaminases were observed within the first months of treatment across all patients: 1.1% for all doses of agomelatine compared with 0.7% for placebo. They were principally observed with agomelatine at a dose of 50 mg per day, for which the crude incidence rate (1.39%) was similar to that documented in comparative studies with venlafaxine at doses of 75–150 mg per day (1.53%) in pooled analyses[44,47] (unpublished data). This led to a recommendation by the EMA of liver function tests as a precautionary measure at the onset of treatment, and then periodically at 6 weeks, 12 weeks and 6 months, as well as subsequently if appropriate. Patients with depression should be regularly followed up by their doctors, so this is less of a constraint than might be initially imagined. In addition, it should be noted that agomelatine is contraindicated in patients with hepatic impairment; for example, cirrhosis or active liver disease.

Finally, sexual dysfunction is a particularly unpopular and frequent side effect of many antidepressants that markedly interferes with quality of life and leads to poor compliance[2,3,107]. Melatonergic agonists and 5-HT$_{2C}$ antagonists actually promote sexual behaviour in animals[2,108–110]. A substantially lower risk of sexual dysfunction with agomelatine treatment compared with venlafaxine treatment was shown in patients at equivalent effective doses[100]. These findings prompted a further investigation that confirmed the good acceptability of agomelatine compared with paroxetine in sexually active healthy volunteers[111].

Leeds sleep evaluation questionnaire
This is a simple and standardized instrument for pseudo-quantifying the influence of therapy on sleep and early morning behaviour. It consists of a number of items like the quality of, and latency to, sleep. The questionnaire is completed by patients themselves.

Discontinuation syndrome
In particular for antidepressants with short half-lives, following long-term (6 weeks or more) treatment, abrupt discontinuation, non-compliance and sometimes even dose reductions can trigger a discontinuation syndrome comprising psychological (agitation, anxiety, irritability) and somatic (nausea, dizziness, sensory and sleep disturbances, flu-like chills, myalgia and fatigue) symptoms. Although usually mild and self-limiting (a week or so), this discontinuation syndrome is distressing and disruptive. Moreover, it can occasionally be quite severe and mistaken for relapse.
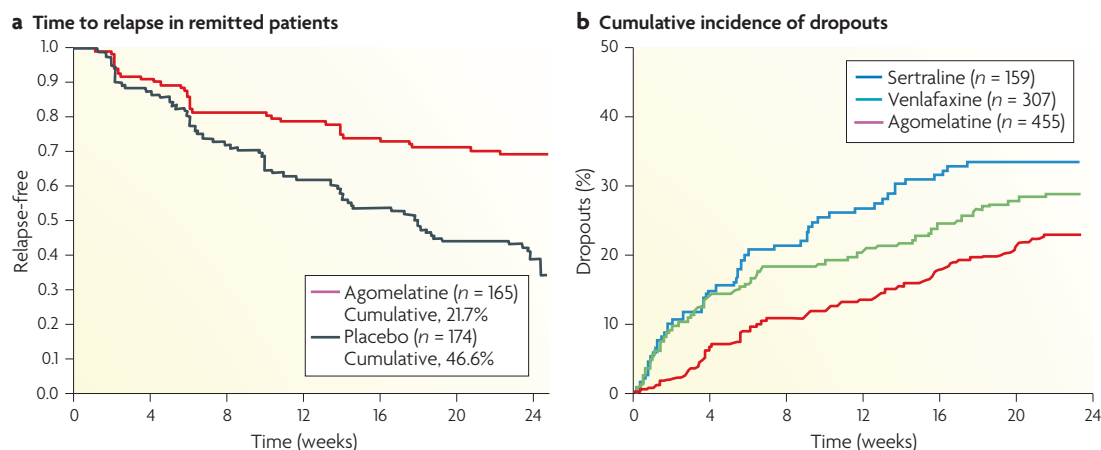
Figure 6 | **Actions of agomelatine in major depression: its prevention of relapse and its low rate of patient dropout compared with comparators. a** | Following treatment with agomelatine, as compared with placebo, there is a marked reduction in the risk for relapse over 6 months. This low rate of relapse has since been shown for up to 10 months [88,97,102]. **b** | The graph depicts the significantly reduced incidence of study dropouts and risk of poor compliance with agomelatine (25–50 mg per day) as compared with the selective serotonin reuptake inhibitor sertraline (50–100 mg per day) and the serotonin/noradrenaline reuptake inhibitor venlafaxine (75–150 mg per day)[99,100].

Looking to the future: the next chapters

Further experimental characterization. Like all other drugs, agomelatine remains a work in progress. Although agomelatine has been thoroughly characterized, there is still much to learn about its mechanism(s) of action and therapeutic promise. Among issues expected to stimulate future research, the following may be emphasized.

First, studies are underway to investigate the respective roles of MT_1 compared with MT_2 receptors in the actions of agomelatine, the significance of (functional or physical) crosstalk among melatonergic and 5-HT_{2C} receptors, and the influence of agomelatine on a host of intracellular signals involved in the pathogenesis of depressed states[19,56,60,112].

Second, agomelatine possesses several complementary mechanisms that potentially favour cognitive performance: enhancement of dopaminergic and adrenergic input to the frontal cortex; increased hippocampal neurogenesis; moderation of stress-induced HPA overactivity; reinforced slow-wave sleep without loss of rapid eye movement sleep; and circadian synchronization[2,113–115]. As cognition is severely impaired in depression[2,116], and agomelatine counters the impairment of cognition by stress[117], it would be interesting to pursue studies of its influence on mnemonic function.

Third, the question arises as to how treatment with agomelatine affects the responsiveness of melatonergic receptors. Studies performed in Chinese hamster ovary cells suggest that human MT_2 receptors are robustly downregulated, decoupled and internalized by melatonin. On the other hand, human MT_1 receptors are more resistant, and night time exposure to high levels of melatonin desensitizes MT_2 but not necessarily MT_1 receptors in rat SCN[40–42]. This lesser tendency of MT_1 receptors to desensitize is intriguing, as they appear to predominate in the SCN of humans[25,29,31]. Long-term regulatory studies of cellular coupling remain to be performed with
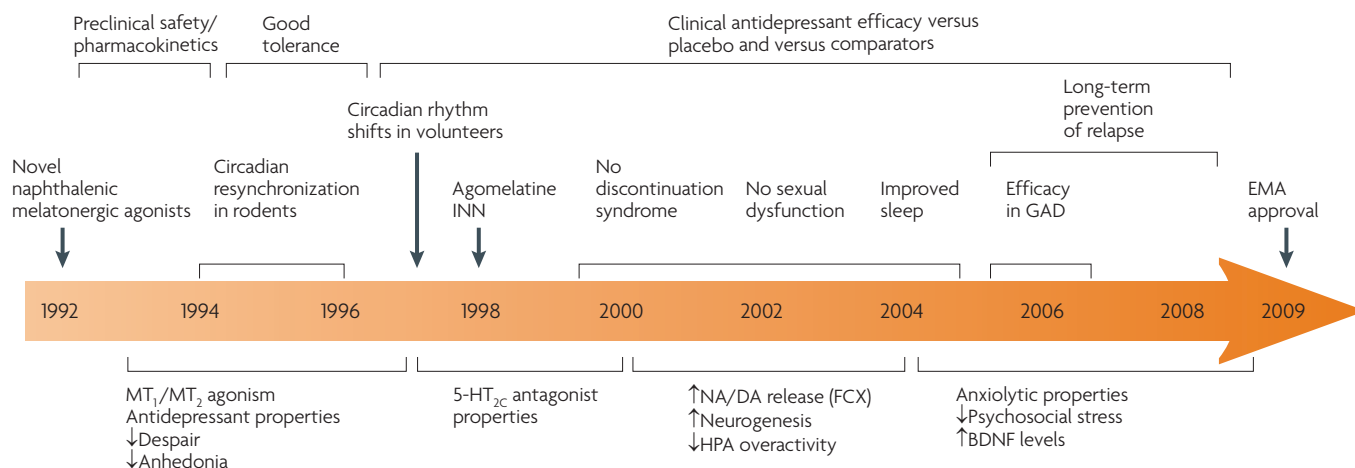
agomelatine, and agonists can differentially affect the sensitivity of G protein-coupled receptors[2], so findings of desensitization with melatonin cannot automatically be extrapolated to agomelatine. Arguing against desensitization, the influence of agomelatine on the activity of SCN neurons in hamsters persisted on repeated administration[43]. Furthermore, the comparatively short half-life of agomelatine mimics the nocturnal pulse of melatonin secretion and should counter downregulation of melatonergic receptors[40,42]. Thus, under conditions of once-daily, night time administration of agomelatine, melatonergic (at least MT_1) receptors in the SCN probably retain their responsiveness, but this issue merits further investigation.

Further clinical characterization. Depression is common but poorly treated in the elderly, who are especially sensitive to distressing side effects of antidepressants and frequently show pronounced perturbations of circadian rhythms[2,118]. Accordingly, in agreement with the EMA, a study of agomelatine is underway[93] in patients over 65 years of age.

One obvious avenue for exploration is seasonal affective disorder, which is characterized by repeated depressive episodes at the same time of year, usually winter[46,119]. Sufferers display disrupted, generally delayed, circadian rhythms and perturbed sleep, as well as alterations in melatonin secretion. Analogous to major depression, melatonin itself does not alleviate seasonal affective disorder (BOX 1), but an open-label study of agomelatine (25 mg per day in the evening) suggested good efficacy over 14 weeks[120], encouraging further controlled studies.

Bipolar disorder is accompanied by a severe disruption of mood and cognition, desynchronization of daily rhythms, and poor sleep[19]. There is evidence for a perturbation of mechanisms controlling neuronal plasticity,

Discovery, development and authorization

Preclinical safety/pharmacokinetics — **Good tolerance** — **Clinical antidepressant efficacy versus placebo and versus comparators**

Circadian rhythm shifts in volunteers

Long-term prevention of relapse

| Novel naphthalenic melatonergic agonists | Circadian resynchronization in rodents | Agomelatine INN | No discontinuation syndrome | No sexual dysfunction | Improved sleep | Efficacy in GAD | EMA approval |

1992 — 1994 — 1996 — 1998 — 2000 — 2002 — 2004 — 2006 — 2008 — 2009

MT$_1$/MT$_2$ agonism
Antidepressant properties
↓Despair
↓Anhedonia

5-HT$_{2C}$ antagonist properties

↑NA/DA release (FCX)
↑Neurogenesis
↓HPA overactivity

Anxiolytic properties
↓Psychosocial stress
↑BDNF levels

Pharmacological characterization: key observations

Figure 7 | **Timeline for the discovery, characterization, development and authorization of agomelatine.** Inherent in a case history is the arrow of time running from conception to discovery and pharmacological characterization to clinical development. This timeline indicates several key phases in the life cycle of agomelatine, culminating in its marketing authorization by the European Medicines Agency (EMA) for the treatment of major depression. However, time has not come to a halt and, in addition to Phase IV clinical surveillance, experimental and clinical exploration of its therapeutic utility continues (see main text). 5-HT, 5-hydroxytryptamine (also known as serotonin); BDNF, brain-derived neurotrophic factor; FCX, frontal cortex; GAD, generalized anxiety disorder; HPA, hypothalamic–pituitary–adrenal; INN, international non-propriety name; MT, melatonin; NA/DA, noradrenaline/dopamine.

such as those that involve glycogen synthase kinase 3β[19], so it is intriguing that agomelatine modulates the activity of this cellular signal[79,112]. Experimental and clinical investigations have been launched to evaluate the potential utility of adjunctive agomelatine in the management of bipolar disorder. In addition, one might explore the adjunctive use of agomelatine in major (unipolar) depression in combination with other antidepressants to improve efficacy and tolerance (such as sleep and sexual function). Nonetheless, the principal benefits of agomelatine are most probably expressed as monotherapy.

The high prevalence of anxious states, and their frequent co-morbidity with depression[2,121], underscores interest in the anxiolytic properties of agomelatine. In a rat model of social defeat, agomelatine mimicked the anxiolytic actions of melatonin, an effect abolished by lesions of the SCN[122]. Furthermore, similar to pharmacological or genetic inactivation of 5-HT$_{2C}$ receptors[66,82,123], agomelatine displayed robust anxiolytic properties (probably expressed in the amygdala and hippocampus) in several procedures in rats[66,124,125]. These observations provided a basis for the evaluation of the potential anxiolytic actions of agomelatine in humans, and the first positive findings in generalized anxiety disorder were recently disclosed[126].

General messages for drug R&D

The history of agomelatine exemplifies several fundamental facets of drug research and development that merit emphasis in a broader context. First, it is crucial that experimental and clinical research is conducted in tandem with development programmes throughout the lifespan of a new compound (FIG. 7). Accordingly, the properties

of drugs must continually be re-evaluated in the light of new developments, from the molecular to the clinical. For example, at the time the agomelatine project was conceived, MT$_1$, MT$_2$ and 5-HT$_{2C}$ receptors had not even been cloned. Second, the discovery of agomelatine reflects a hypothesis-driven process of research founded on the notion that depression is not a monolithic disorder of mood but displays, among other factors, a disruption of biological rhythms; this concept continues to guide its characterization. Third, it is impossible to anticipate everything from the outset. As good fortune favours the prepared mind (and laboratory), flexibility and reactivity are crucial elements for successful research and development programmes. Accordingly, the unexpected 5-HT$_{2C}$ receptor antagonist properties of agomelatine were systematically validated as complementary to its melatonin agonist actions. Finally, a huge, long-term and risk-intensive effort from inception to market authorization was needed to realize the objective of making agomelatine available to patients suffering from major depression (FIG. 7).

Concluding comments

Agomelatine represents an innovative approach to treating depression[73] as it is the first regulatory approved agent to incorporate a non-monoaminergic mechanism. Its antidepressant activity across a broad range of experimental procedures in animal models and its distinctive therapeutic profile in humans probably reflect a synergistic interplay of its melatonergic (agonist) and 5-HT$_{2C}$ (antagonist) properties. Extensive clinical trials have established both the short-term and long-term efficacy of agomelatine in major depression in mildly

and severely ill patients, with an improvement of sleep quality, preservation of sexual function, absence of weight gain and good tolerability. Moreover, discontinuation is not associated with withdrawal symptoms. This overall profile compares favourably to currently available antidepressants and should encourage adherence throughout the extensive treatment period, 6 months or more, recommended for durable improvement of a major depressive episode. Further studies and clinical use will clarify the full potential of agomelatine as an antidepressant. There is also rich potential, as highlighted above, for exploration of the broader use of agomelatine in the treatment of other central nervous system disorders.

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DATABASES
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http://www.ncbi.nlm.nih.gov/omim
Depression
IUPHAR Database of Receptors and Ion Channels:
http://www.iuphar-db.org
5-HT$_{2C}$ | MT$_1$ | MT$_2$

FURTHER INFORMATION
European Public Assessment Report — Valdoxan:
http://www.emea.europa.eu/humandocs/PDFs/EPAR/valdoxan/H-915-en6.pdf

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