Drug discovery: phosphinolactone, *in vivo* bioisostere of the lactol group†

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In drug discovery, structural modifications over the lead molecule are often crucial for the development of a drug. Herein, we reported the first *in vivo* bioisosteric effect of phosphinolactone function in relation to the lactol group constituting the bioactive molecule: Hydroxybupropion. The preparation of phosphinolactone analogues and their antidepressant evaluation towards forced swimming test in mice showed that biological activity was regained and even strengthen.

Introduction

A critical issue for innovation in drug discovery is the search for new pharmacophores or bioisosteric groups in order to modulate the metabolic and/or the pharmacokinetic properties of drugs. Therefore, finding analogy for biologically stable and patentable modules would be a very desirable pivotal step on the way to increase the success rate of screening.[1]

Depression is a frequent and common neurological psychiatric disorders from adulthood.[2,3] A study carried out in the USA in 1994, estimated that the lifetime prevalence for major depression was 17.1% in persons aged between 15–54 years.[3] Although a «therapeutic arsenal» is available to treat depressive patients, a significant number of them will reveal insensitive or will have inadequate responses to the usable treatments. This percentage has been recently evaluated to about 38%.[4] In addition, the economic impact of this illness in our occidental societies is far from insignificant. Therefore, development of new antidepressants still continues to be a challenge in order to substitute inefficient molecules. Another societal thorny problem concerns tabagism and its damage on health. In the USA, a vast majority of smokers (70%) would like to quit smoking,[5] but at the present time, smoking-cessation treatments do not entail a decrease in the number of people having a smoking addiction. It also becomes obvious that discovery of innovative therapeutic agents is a major issue. To date, Bupropion **1** (Fig. 1), marketed in 1989 by GlaxoSmithKline in the USA is an atypical compound which has been successfully used both as antidepressant (Wellbutrin) and as smoking-cessation aid (Zyban). It has been reported to act as a moderate inhibitor of dopamine (DA) and noradrenaline (NE) reuptake, with *in vitro* IC_{50} values of 550 nM and 1.9 μM, respectively, in rat cortical synaptosomes preparations.[5] In fact, it has been proposed that Bupropion **1** activity would come of its active metabolite, (2*S*,3*S*)-Hydroxybupropion **2** (Radafaxine). Indeed, this compound exhibits a similar activity on DA uptake systems but a better inhibition of NE transporters, with IC_{50} values of 790 nM and 520 nM, respectively.[5] Moreover, hydroxybupropion **2** offers a better bioavailability, with higher plasma concentration in human than its parent compound, in line with its involvement in the mechanism of action of Bupropion.[6] Moreover, it has been underlined that Bupropion **1** and its metabolite (2*S*, 3*S*)-hydroxybupropion **2** also act on nicotinic acetylcholine receptor (nAChR), an effect supposed to be related in its smoking cessation efficacy.[5] Together, these results suggest that the pharmacological effects of Bupropion **1** could arise from simultaneous actions at these different targets, and that its efficacy benefits from its active metabolite (2*S*, 3*S*)-hydroxybupropion.

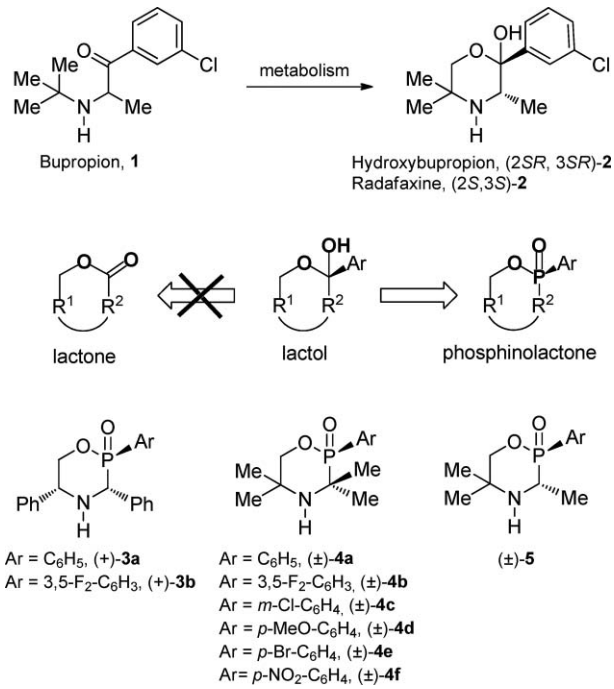

Fig. 1 Analogy between lactol and phosphinolactone group.

We suspected an analogy between the lactol group and phosphinolactone with a close correspondence of these two structures both in term of polarity and possibly biological activity (Fig. 1).

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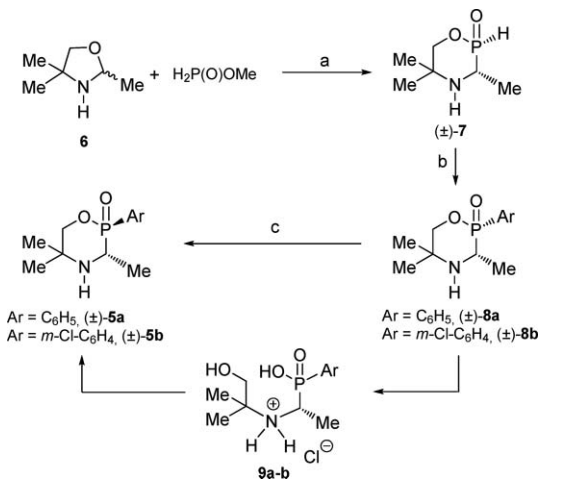
From a structural point of view, the tetrahedral geometry of the phosphinolactone group can be directly addressed as a mime of the hemiketal function. In contrast, the sp^2 hybridized ester group of lactone sugar ring possesses a partial analogy with lactol and therefore it can be considered only as an imperfect bioisostere.

We already showed that phostine, another phosphinosugar family, standing half-way between C-aryl-glycosides and phosphonosugars, with also phosphinolactone subunit for the antiproliferative effect[7] is able to inhibit cell glycosylation (to be published).

The analysis of calculated Van der Waals volumes suggests that the phosphinolactone unit occupies nearly the same volume as the hemiketal group. Another critical and essential physical parameter for neuroactive compounds is the diffusion through the blood–brain barrier (BBB). Physicochemical properties notably influence BBB permeability, a preferred range brain penetration occurs with compounds possessing Log P ~ 2–4.[8] The estimated value of the phosphinyl analogue **5** (clogP = 2.81)[9] is revealed similar to the calculated LogP of hydroxybupropion **2** (clogP = 2.74,[9] published 2.87).[10]

In this context, we were interested in an amenable and convenient synthesis of intracyclic phosphinate compounds. To validate the relevance of our hypothesis, we choose to incorporate such group in compounds of pharmacological interest such as **2** and related arylmorpholinol.[11,12] As a continuation of our synthetic studies for preparation of original [1,4,2]-oxazaphosphinanes **3**[13] and **4**[14] (Fig. 1), we report herein the preparation of exact analogues in phosphinyl series **5** of the (2*SR*, 3*SR*)-hydroxybupropion **2** and their *in vivo* evaluation as antidepressant in mice.

A synthetic route was designed to allow the construction and subsequent elaboration of diversified structures for an *in vivo* screening (Pathway A). The preparation of oxazaphosphinanes **5** exhibiting *trans* configuration was planned according to a methodology previously developed for **3**[13] and **4**,[14] which of the final key step of the chemical pathway is the non-classical and selective epimerization of the phosphorus atom[13] (Scheme 1). The first step consisted in a condensation of methyl hypophosphite with 2,4,4-trimethyl-1,3-oxazolidine **6** to give the H-oxazaphosphinane **7**. An excess of corresponding oxazolidine **6** was necessary to entirely consume methyl hypophosphite and thus to optimize the formation of the oxazaphosphinane **7** in a 45% yield (evaluated by ^{31}P NMR). Oxazolidine **6** was previously obtained by a simple condensation of acetaldehyde with 2-methyl-2-aminopropanol as proposed by Haynes and Philips,[15] and phosphinate was prepared *in situ* according to a slightly modified procedure described by our research group.[16] After chromatography of the crude, compound **7** was isolated in an 18% yield. Following, P–H function was arylated through a pallado-catalyzed reaction using common conditions.[13,14] Thus compounds **8a** and **8b** were obtained as racemic mixtures respectively in 71% and 43% yields. Geometry and relative stereochemistry of **8** and in consequence **7** were determined from X-ray diffraction studies of **8b**, obtained after crystallization in diethyl ether/hexanes. As for the tetramethyl analogue,[14] the trimethyl six-membered ring adopts the expected chair conformation with the *meta*-chlorophenyl group in axial position and the phosphinyl P=O bond in equatorial position (Fig. 2).[17]



Scheme 1 Pathway A for preparation of **5**. *Reagents and conditions*: a) THF–toluene (1/1), 0 °C → RT, 18%; b) ArX, $Pd(PPh_3)_4$, Et_3N, toluene, 70 °C, 4 h, (ArX = C_6H_5I, (±)-**8a**, 71%) and (ArX = *m*-Cl–C_6H_4Br, (±)-**8b**, 43%); c) HCl (35%), 50 °C, 3 days, thus NaOH until pH = 9, (±)-**5a**, 70% and (±)-**5b**, 74%.

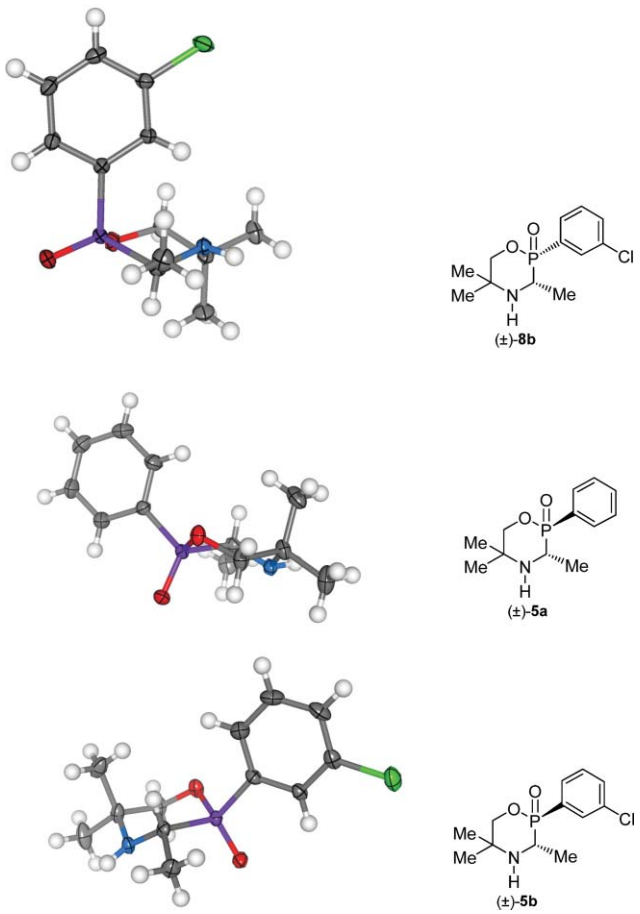
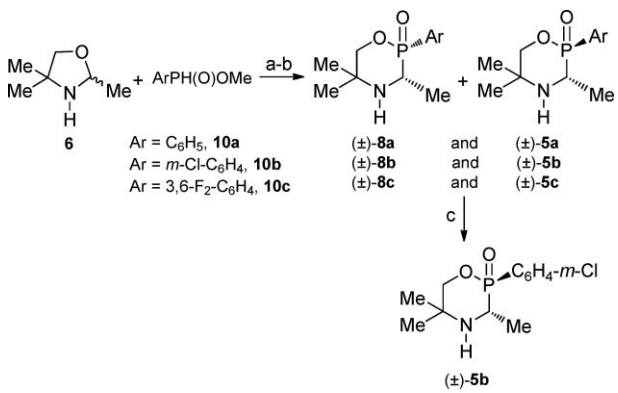


Fig. 2 Chair conformation as determined X-ray diffraction of (±)-**8b**, (±)-**5a** and (±)-**5b**. Atomic displacement ellipsoids were drawn at the 50% probability level.

Afterwards, epimerization reaction of **8a** and **8b** in strong acidic media[13] gave with success epimers **5a** and **5b** (Scheme 1). The behaviour of the reaction was studied on the basis of ^{31}P NMR spectra. The evolution of **8b** showed that the *cis* structure

was partially consumed after 2 h at 50 °C (δ = 33.4 ppm, 23% remained) and transformed into the *trans* isomer **5b** (δ = 32.1 ppm, 13%). Besides the starting material and its epimer, another compound **9b** (36.8 ppm, 64%) was characterized by high resolution mass spectrometry. After one day at 50 °C, only 2–3% of **8b** remained and 80% of epimer **5b** along with 16% of phosphinic intermediate **9b** were obtained. Finally, another period of two days heating furnished a final ratio **5b**/**9b** of 9/1. Pure *trans* oxazaphosphinane **5b** placed under strong acidic conditions during 3 days [HCl (35%), 50 °C], gave the same ratio 9/1 in **5b** and **9b**, proving thus the reversible character of this reaction. Pure compounds **5a** and **5b** were readily recovered after simple alkalinisation and extraction from the aqueous media. Their *trans* configurations were confirmed by an X-ray experiment from the corresponding single crystal prepared by recrystallization in diethyl ether/hexanes. In both cases, the six-membered ring adopts a chair conformation and the phenyl or *meta*-chlorophenyl group occupies the equatorial position whereas the phosphinyl bond is in axial position (Fig. 2).[17] Configuration and conformation of the ammonium salt of (2*S*, 3*S*)-Radafaxine **2** revealed very same to **5b**.[18]

In parallel, in order to evade the difficult preparation of the hydrogeno-oxazaphosphinane **7**, a shorter reaction pathway B has been investigated (Scheme 2). Instead of methyl hypophosphite as starting material, arylphosphinate methyl esters **10** were used. These precursors such as methyl 3-chloro-phenyl, 3,5-difluoro-phenyl and phenylphosphinates, were prepared by methylation of corresponding phosphinic acids derivatives with methyl chloroformate, applying a similar procedure described by Afarinkia.[19] When H-phosphinic acids or esters are not commercially available, they are obtained by pallado-catalyzed arylation of the anilinium hypophosphite, according to methodology reported by Montchamp.[20]



Scheme 2 Pathway B for preparation of **5**. *Reagents and conditions*: a) ArPH(O)OMe **10a–c**, THF–toluene (1/1), 24 h, 50 °C; b) *t*-BuOK (cat.), −5 °C → RT, (±)-**8a**, 29% and **5a**, 33%, (±)-**8b**, 44% and **5b**, 48%, (±)-**8c**, 35% and **5a**, 38%; c) HCl (35%), 50 °C, 3 days, thus NaOH until p*H* = 9, (±)-**5b**, 65% (from oxazolidine **6**).

Arylphosphinates **10a–c** were reacted with an excess of oxazolidine **6** (2 equivalents) at 50 °C. After 24 h, catalytic amounts of potassium *tert*-butoxide were added into the reaction mixture to reach the completion in *cis* and *trans* oxazaphosphinanes **8a–c**/**5a–c** without any stereoselectivity and a ^{31}P NMR ratio **8a–c**/**5a–c** close to 50 : 50. *Cis* and *trans* diastereomers were readily separated by chromatography on silica, and yields are reported in the Scheme 2. Moreover, a direct epimerization of the crude mixture **8b**/**5b** has been led with success, affording the formation of thermodynamic compound **5b** in 65% overall yield based on oxazolidine **6**.

Oxazaphosphinanes and reference compounds **1** and **2** were, in a first intent, screened for their biological activity in the forced swimming test in mice, a choice model to delineate antidepressant-like activity in rodents.[21] The drugs were tested at the dose of 20 mg kg^{-1} i.p, and as shown in Fig. 3a, the ANOVA analysis ($F_{(11,108)} = 3.45$, $p < 0.001$) revealed that, among the compounds tested, only **5c** and **4c** significantly reduced the immobility duration, by −34% and −44% respectively. These effects were slightly lower or comparable to the effect induced by bupropion (−52%) or (2*SR*, 3*SR*)-hydroxybupropion (−54%).

The dose-response effect of the two reference compounds, bupropion and (2*SR*, 3*SR*)-hydroxybupropion and the two active compounds, **4c** and **5c**, were examined in the 0–40 mg kg^{-1} dose range. Bupropion showed a monophasic dose-response curve, with significant reduction of the immobility duration at 20 and 40 mg kg^{-1} ($F_{(3,32)} = 6.55$, $p < 0.01$; Fig. 3b). (2*SR*, 3*SR*)-hydroxybupropion showed a biphasic curve, with significant reduction of the immobility duration at 20 and 40 mg kg^{-1} ($F_{(3,33)} = 8.95$, $p < 0.001$; Fig. 3c), the most significant effect being measured at 20 mg kg^{-1}. **4c** also showed a biphasic curve, with significant reduction of the immobility duration at 10 and 20 mg kg^{-1} ($F_{(4,46)} = 3.55$, $p < 0.05$; Fig. 3d), the most significant effect being measured at 10 mg kg^{-1}. **5c** showed a similar profile, with significant effects at 10 and 20 mg kg^{-1} ($F_{(4,51)} = 2.70$, $p < 0.05$; Fig. 3e).

These behavioral data demonstrated the efficacy of both compounds **4c** and **5c** *in vivo*. Particularly, **4c** at its maximum effect could be considered as four fold more potent than bupropion **1** and twice more potent than (2*SR*, 3*SR*)-hydroxybupropion **2**.

Conclusions

Phosphinolactone is easily introduced in replacement of the lactol group of (2*SR*, 3*SR*)-hydroxybupropion leading to new active compounds. Thus, phosphinolactone group might be considered as a bioisostere of lactol group and used as an unprecedented scaffold for the elaboration of new candidate drugs. Chemical and stereochemical stability in basic or acidic media, similar steric hindrance and polarity illustrate the usefulness of this original structural core. Another outstanding result is the ability of 1,4,2-oxazaphosphinanes to diffuse through the blood–brain barrier in mice to reproduce the original activity observed with Bupropion **1** or with (2*SR*, 3*SR*)-hydroxybupropion **2** at lower concentration.

Experimental

General experimental techniques

Chemistry. Before use, commercial reagents were purified by distillation or sublimation. Tetrakis(triphenylphosphine)-palladium(0) was stored under nitrogen and used without any other purification. Solvents were dried according to current methods, distilled and stored under nitrogen atmosphere. All reactions involving air or moisture sensitive reagents or intermediates were carried out under dry nitrogen in flame-dried glassware. Melting

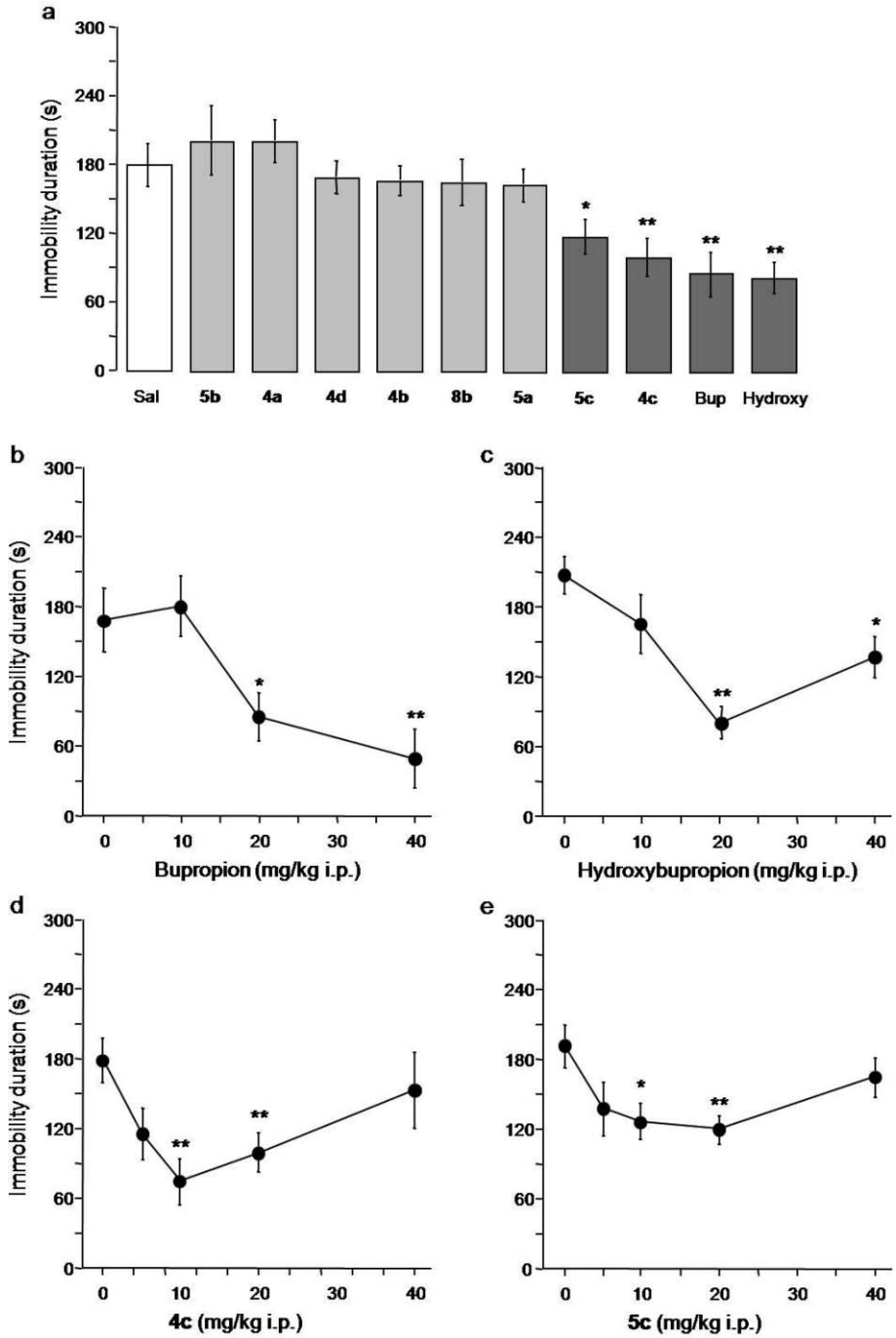


Fig. 3 (**a**) Screening of the antidepressant activity of the compounds in the forced swimming test in mice. All compounds were injected at 20 mg kg^{-1} i.p. 30 min before the second session of the test. $n = 6$–18 per group. Dose-response effects (0–40 mg kg^{-1} i.p.): (**b**) bupropion, $n = 7$–10 mice per group. (**c**) (2*SR*, 3*SR*)-hydroxybupropion, $n = 8$–10. (**d**) **4c**, $n = 8$–12. (**e**) **5c**, $n = 7$–21. * $p < 0.05$, ** $p < 0.01$ *vs.* saline-treated group; Dunnett's test.

points were measured on a Büchi B-540 apparatus and are uncorrected. Spectrums ^{1}H, ^{19}F, ^{31}P and ^{13}C were recorded on a Bruker DRX 400 MHz spectrometer operating respectively at frequency of 400 MHz, 188 MHz, 162 MHz and 101 MHz. All NMR experiments performed on phosphorus are indicated uncoupling of hydrogen and all studies led during processing were done with a DMSO-D_6 probe. High resolution mass spectra were measured on JEOL JMS–SX 102A spectrometer (ion mode FAB+) or on Micromass QTOF (ion mode ES+). X-Ray data were collected on an Oxford-Diffraction Xcalibur-I diffractometer using graphite monochromated Mo-radiation.

Pharmacology. Male Swiss OF1 mice (Depré, Saint Doulchard, France), aged 6–8 weeks and weighing 28 ± 2 g were used. Animals were housed in groups with free access to laboratory chow and water in a regulated environment (22 ± 1 °C,

40–60% humidity) and under a 12 h/12 h light/dark cycle (light on at 7 : 00 AM). Experiments were carried out between 1 : 00 PM and 4 : 00 PM. All animal procedures were conducted in strict adherence of European Council Directives of 24 November 1986 (86–609). Bupropion was from Sigma-Aldrich (Saint Quentin-Fallavier, France) and (2*SR*, 3*SR*)-hydroxybupropion was from Becton Dickinson (Le-Pont-de-Claix, France). Compounds, solubilized in physiologic saline solution, were injected intraperitoneally (i.p.) in a volume of 100 µl per 20 g of body weight.

2,4,4-Trimethyloxazolidine 6

2,4,4-Trimethyloxazolidine (**6**) was prepared according to the original procedure described by Haynes and Philips;[15] δ_H ($CDCl_3$): 1.13 (3 H, s, O–C–C*H*$_3$), 1.21 (3H, s, O–C-CH$_3$), 1.30 (3H, d, $^3J_{HH}$ = 5.5 Hz, CH–C*H*$_3$), 1.61 (1H, br), 3.36 (1H, d, $^2J_{HH}$ = 7.6 Hz, O–C*H*$_2$), 3.42 (1H, d, $^2J_{HH}$ = 7.6 Hz, O–C*H*$_2$), 4.56 (1H, q, $^3J_{HH}$ = 5.3 Hz, C*H*-CH$_3$); ^{13}C ($CDCl_3$): δ_C 20.1 (s, CH-*C*H$_3$), 26.6 (s, CH$_2$–C-*C*H$_3$), 27.3 (s, CH$_2$–C-*C*H$_3$), 77.4 (s, *C*H$_2$), 87.83 (s, *C*H).

(±)-3,5,5-Trimethyl-2-hydrogeno-2-oxo-[1,4,2]-oxazaphosphinane 7

In a 100 mL two-necked flask containing methyl hypophosphite [(60 mmol, prepared according to a slight modification of the procedure described in literature,[16] mixing hypophosphorous acid (4.0 g, 60 mmol) and trimethyl orthoformate (28.7 g, 29.5 mL, 270 mmol)] in a mixture THF–toluene (30 mL, 1 : 1) was added 2,4,4-trimethyloxazolidine (13.6 g, 120 mmol) to 0 °C. The mixture was stirred 2 h, thus solvents were removed by evaporation and the residue dried with a high vacuum and purified by column chromatography on silica gel (45–60 µm) with ethyl acetate–ethanol as eluent (100/0 to 90/10). After concentration, pure compound **7** was obtained as a white solid (18%); mp = 69–70 °C; δ_P ($CDCl_3$): 39.51 (s); δ_H ($CDCl_3$): 1.02 (3H, s, CH$_2$–C–C*H*$_3$), 1.22 (3H, dd, $^3J_{PH}$ = 21.2 Hz, $^3J_{HH}$ = 7.1 Hz, P-CH–C*H*$_3$), 1.31 (3H, s, CH$_2$–C–C*H*$_3$), 3.23 (1H, ddd, $^2J_{PH}$ = 8.6 Hz, $^3J_{HH}$ = 7.05 Hz, $^3J_{HH}$ = 5.9 Hz, P–C*H*-CH$_3$), 3.87 (1H, dd, $^3J_{PH}$ = 19.1 Hz, $^2J_{HH}$ = 11.1 Hz, C*H*$_2$-O), 3.97 (1H, dd, $^3J_{PH}$ = 4.8 Hz, $^2J_{HH}$ = 11.1 Hz, C*H*$_2$-O), 6.56 (1H, dd, $^1J_{PH}$ = 522.3 Hz, $^2J_{PH}$ = 6.0 Hz, P(O)*H*); δ_C ($CDCl_3$): 14.2 (d, $^2J_{PC}$ = 2.2 Hz, P-CH-*C*H$_3$), 22.4 (s, CH$_2$–C-*C*H$_3$), 26.1 (s, CH$_2$–C-*C*H$_3$), 45.8 (d, $^1J_{PC}$ = 89.3 Hz, P-*C*H-CH$_3$), 50.8 (d, $^3J_{PC}$ = 5.1 Hz, CH$_2$-*C*-CH$_3$), 79.3 (d, $^2J_{PC}$ = 9.5 Hz, O-*C*H$_2$); HRMS (FAB$^+$) MH$^+$ calcd $C_6H_{15}NO_2P$: 164.0840; found 164.0856.

Pathway A. General procedure for the preparation of 2-aryl-3,5,5-trimethyl-2-oxo-[1,4,2]-oxazaphosphinanes (8a–b)

In a 25 mL Schlenk tube containing 3,5,5-trimethyl-2-hydrogeno-2-oxo-[1,4,2]-oxazaphosphinane (**7**, 0.49 g, 3.0 mmol) under nitrogen at room temperature were successively added, toluene (2.0 mL), aryl iodide (3.3 mmol), triethylamine (9 mmol, 1.3 mL), and palladium tetrakis(triphenyl)phosphine (0.17 g, 0.15 mmol). The reaction mixture was stirred and heated at 70 °C for 4 h. After cooling, the mixture was concentrated under vacuum and the crude residue was purified by column chromatography on silica gel with ethyl acetate–ethanol (100/0 to 90/10) as eluent. After concentration, pure compounds **10a–b** were obtained.

(±)-2-Phenyl-3,5,5-trimethyl-2-oxo-[1,4,2]-oxazaphosphinane 8a. (71%); mp = 106–108 °C. δ_P ($CDCl_3$): 37.78 (s). δ_H ($CDCl_3$): 1.08 (3H, dd, $^3J_{PH}$ = 18.2 Hz, $^3J_{HH}$ = 7.1 Hz, P-CH–C*H*$_3$), 1.16 (3H, s, CH$_2$–C–C*H*$_3$), 1.27 (3H, s, CH$_2$–C–C*H*$_3$), 3.47 (1H, qd, $^3J_{PH}$ = 11.6 Hz, $^3J_{HH}$ = 7.1 Hz, P–C*H*-CH$_3$), 4.00 (2H, d, $^2J_{HH}$ = 12.4 Hz, O–C*H*$_2$), 7.40–7.46 (2H, m, C*H*ar), 7.50–7.97 (1H, m, C*H*ar), 7.90–8.10 (2H, m, C*H*ar). δ_C ($CDCl_3$): 15.7 (s, P-CH-*C*H$_3$), 23.5 (s, CH$_2$–C-*C*H$_3$), 27.3 (s, CH$_2$–C-*C*H$_3$), 47.1 (d, $^1J_{PC}$ = 98.8 Hz, N-*C*H–P), 51.5 (d, $^3J_{PC}$ = 1.5 Hz, CH$_2$-*C*-CH$_3$), 74.8 (d, $^2J_{PC}$ = 7.3 Hz, O-*C*H$_2$), 127.5 (d, $^1J_{PC}$ = 122.2 Hz, *C*ar), 128.4 (d, $^3J_{PC}$ = 12.4 Hz, *C*ar), 132.5 (d, $^2J_{PC}$ = 8.8 Hz, *C*Har), 132.7 (d, $^4J_{PC}$ = 2.9 Hz, *C*Har); HRMS (FAB$^+$) MH$^+$ calcd $C_{12}H_{19}NO_2P$: 240.1153; found 240.1155.

(±)-2-(3-Chlorophenyl)-3,5,5-trimethyl-2-oxo-[1,4,2]-oxazaphosphinane 8b. (44%); mp = 113–114 °C. δ_P ($CDCl_3$): 35.52 (s). δ_H ($CDCl_3$): 1.19 (3H, dd, $^3J_{PH}$ = 18.7 Hz, $^3J_{HH}$ = 7.0 Hz, P-CH–C*H*$_3$), 1.24 (3H, s, CH$_2$–C–C*H*$_3$), 1.38 (3H, s, CH$_2$–C–C*H*$_3$), 3.60 (1H, dq, $^2J_{PH}$ = 11.6 Hz, $^3J_{HH}$ = 7.0 Hz, N–C*H*-CH$_3$), 4.06 (1H, dd, $^3J_{PH}$ = 13.9 Hz, $^2J_{PH}$ = 11.6 Hz, O–C*H*$_2$-CH$_3$), 4.10 (1H, dd, $^2J_{HH}$ = 11.6 Hz, $^3J_{PH}$ = 9.9 Hz, O–C*H*$_2$-CH$_3$), 7.40–7.50 (1H, m, C*H*ar), 7.55–7.60 (1H, m, C*H*ar), 7.95–8.05 (1H, m, C*H*ar), 8.05–8.15 (1H, m, C*H*ar). δ_C ($CDCl_3$): 15.6 (s, P-CH-*C*H$_3$), 23.4 (s, CH$_2$–C-*C*H$_3$), 27.2 (s, CH$_2$–C-*C*H$_3$), 47.3 (d, $^1J_{PC}$ = 101.0 Hz, P-*C*H-CH$_3$), 51.6 (d, $^3J_{PC}$ = 2.2 Hz, CH$_2$-*C*-CH$_3$), 75.4 (d, $^2J_{PC}$ = 7.3 Hz, O-*C*H$_2$), 129.9 (d, $^3J_{PC}$ = 13.2 Hz, CHar), 129.9 (d, $^1J_{PC}$ = 119.3 Hz, P-*C*ar), 130.6 (d, $^2J_{PC}$ = 8.1 Hz, *C*Har), 132.3 (d, $^2J_{PC}$ = 9.5 Hz, *C*Har), 132.8 (d, $^4J_{PC}$ = 2.9 Hz, *C*Har), 134.8 (d, $^3J_{PC}$ = 16.1 Hz, *C*ar-Cl); HRMS (FAB$^+$) MH$^+$ calcd $C_{12}H_{18}ClNO_2P$: 274.0764; found 274.0768.

Preparation of methyl arylphosphinates (10a–c)

Methyl arylphosphinate esters were accessible by methylation from corresponding acid derivatives according to methodology developed by Afarinkia.[19] Phosphinic acids such as 3-chloro- and 3,5-difluorophenyl-phosphinic acids were prepared using the same strategy described by Montchamp.[20] Phenylphosphinic acid is commercially available.

3-Chlorophenylphosphinic acid[20,22]. (53%); for data see references 20 and 22.

3,5-Difluorophenylphosphinic acid. (22%); mp = 85–86 °C. δ_P ($CDCl_3$): 19.56 (t, $^3J_{PF}$ = 7.9 Hz). δ_H ($CDCl_3$): 6.9–6.7 (1H, m, C*Har*), 7.0–7.2 (2H, m, C*Har*), 7.34 (1H, d, $^1J_{PH}$ = 587.4, P-*H*); HRMS (FAB$^+$) MH$^+$ calcd $C_6H_6F_2O_2P$

Methyl 3-phenylphosphinate 10a[19,22,23]. For preparation, see ref. 19 and for data, see ref. 19, 22 and 23.

Methyl 3-chlorophenylphosphinate 10b[22]. (53%); δ_P ($CDCl_3$): 24.7 (s). δ_H ($CDCl_3$): 3.75 (3H, d, $^3J_{PH}$ = 12.1 Hz, O–C*H*$_3$), 7.41 (1H, td, *J* = 7.8, 3.8 Hz, C*Har*), 7.52 (1H, dm, *J* = 7.8 Hz, C*Har*), 7.61 (1H, ddt, *J* = 13.4, 7.6, 1.3 Hz, C*Har*), 7.48 (1H, d, $^1J_{PH}$ = 573.7 Hz, P-*H*), 7.70 (1H, dt, *J* = 13.9, 1.5 Hz, C*Har*).

Methyl 3,5-difluorophenylphosphinate 10c. (92%); δ_P ($CDCl_3$): 22.97 (t, $^4J_{PF}$ = 7.9 Hz). δ_H ($CDCl_3$): 3.85 (3H, d, $^3J_{PH}$ = 12.1 Hz, O–C*H*$_3$), 7.0–7.2 (1H, tt, *J* = 8.6, 2.3, C*Har*), 7.2–7.5 (2H, m, C*Har*), 7.56 (1H, d, $^1J_{PH}$ = 581.3 Hz, P-*H*); HRMS (ES$^+$) MH$^+$ calcd $C_7H_8F_2O_2P$: 193.0230; found 193.0233.

Pathway B. General procedure for preparation of 2-aryl-2-oxo-[1,4,2]-oxazaphosphinanes in mixture *cis* (8a–c) and *trans* (5a–c) forms from methyl arylphosphinates

2,2,4-Trimethyloxazolidine (**8**, 20 mmol, 2.3 g) and arylphosphinate (10 mmol) in THF–toluene (10 mL, 1 : 1) was heated to 50 °C for 24 h (15 h for **10b/11b**). Then to −5 °C, *t*-BuOK (1 mmol, 0.12 g) was added and the mixture was raised to ambient temperature. After 1 h, solvents were evaporated and two diastereomers (*cis*/*trans*) were separated by column chromatography on silica gel with ethyl acetate–ethanol as eluent (100/0 to 90/10). Compounds with *cis* configuration **10a–c**, thus those with *trans* configuration **11a–c** were successively recovered pure.

(±)-2-Phenyl-3,5,5-trimethyl-2-oxo-[1,4,2]-oxazaphosphinane 8a. (*cis* form, 29%); data see pathway A and **5a** (*trans* form, 33%); mp = 142–143 °C; δ_P ($CDCl_3$): 31.62 (s); δ_H ($CDCl_3$): 1.09 (3H, s, CH_2–C–CH_3), 1.15 (3H, dd, $^3J_{PH}$ = 16.9 Hz, $^3J_{HH}$ = 7.0 Hz, P-CH–CH_3), 1.39 (3H, s, CH_2–C–CH_3), 3.24 (1H, qd, $^3J_{HH}$ = 6.9 Hz, $^2J_{PH}$ = 4.6 Hz, P–C*H*-CH_3), 3.86 (1H, dd, $^3J_{PH}$ = 16.4 Hz, $^2J_{HH}$ = 11.2 Hz, O–CH_2), 4.36 (1H, dd, $^3J_{PH}$ = 3.0 Hz, $^2J_{HH}$ = 11.2 Hz, O–CH_2), 7.40–7.50 (2H, m, C*H*ar), 7.50–7.60 (1H, m, C*H*ar), 7.75–7.85 (2H, m, C*H*ar); δ_C ($CDCl_3$): 14.9 (s, P-CH-*C*H_3), 22.4 (s, CH_2–C-*C*H_3), 26.4 (s, CH_2–C-*C*H_3), 46.0 (d, $^1J_{PC}$ = 91.5 Hz, N-*C*H–P), 50.8 (d, $^3J_{PC}$ = 3.7 Hz, CH_2-*C*-CH_3), 74.2 (d, $^2J_{PC}$ = 5.3 Hz, O-*C*H_2), 128.5 (d, $^1J_{PC}$ = 125.1 Hz, *C*ar), 128.7 (d, $^3J_{PC}$ = 12.4 Hz, *C*ar), 131.7 (d, $^2J_{PC}$ = 10.2 Hz, *C*Har), 132.9 (d, $^4J_{PC}$ = 2.2 Hz, *C*Har); *m*/*z* (ES^+) MH^+ calcd $C_{12}H_{19}NO_2P$: 240.1153; found 240.1124.

(±)-2-(3-Chlorophenyl)-3,5,5-trimethyl-2-oxo-[1,4,2]-oxazaphosphinane 8b. (*cis* form, 32%); data see pathway A and **5b** (*trans* form, 36%); mp = 130–131 °C; δ_P ($CDCl_3$): 30.16 (s). δ_H ($CDCl_3$): 1.15 (3H, s, CH_2–C–CH_3), 1.22 (3H, dd, $^3J_{PH}$ = 17.2 Hz, $^3J_{HH}$ = 7.1 Hz, P-CH–CH_3), 1.37 (3H, s, CH_2–C–CH_3), 3.21 (1H, qd, $^3J_{HH}$ = 7.0 Hz, $^2J_{PH}$ = 4.9 Hz, N–C*H*-CH_3), 3.96 (1H, dd, $^3J_{PH}$ = 16.1 Hz, $^2J_{HH}$ = 11.1 Hz, O–CH_2), 4.41 (1H, dd, $^2J_{HH}$ = 11.1 Hz, $^3J_{PH}$ = 3.0 Hz, O–CH_2), 7.40–7.50 (1H, m, C*H*ar), 7.55–7.60 (1H, m, C*H*ar), 7.70–7.80 (1H, m, C*H*ar), 7.80–7.90 (1H, m, C*H*ar). δ_C ($CDCl_3$): 14.8 (s, P-CH-*C*H_3), 22.4 (s, CH_2–C-*C*H_3), 26.3 (s, CH_2–C-*C*H_3), 46.0 (d, $^1J_{PC}$ = 92.2 Hz, P-*C*H-CH_3), 50.8 (d, $^3J_{PC}$ = 4.4 Hz, CH_2-*C*-CH_3), 74.3 (d, $^2J_{PC}$ = 5.1 Hz, O-*C*H_2), 129.8 (d, $^2J_{PC}$ = 9.5 Hz, *C*Har), 130.1 (d, $^3J_{PC}$ = 13.2 Hz, *C*Har), 130.8 (d, $^1J_{PC}$ = 122.2 Hz, *C*ar), 131.5 (d, $^2J_{PC}$ = 11.0 Hz, *C*Har), 132.9 (d, $^4J_{PC}$ = 2.2 Hz, *C*Har), 135.0 (d, $^1J_{PC}$ = 16.1 Hz, *C*ar-Cl); HRMS (ES^+) MH^+ calcd $C_{12}H_{18}ClNO_2P$: 274.0764; found 274.0787.

(±)-2-(3,5-Difluorophenyl)-3,3,5,5-tetramethyl-2-oxo-[1,4,2]-oxazaphosphinane 8c. (*cis* form, 35%); mp = 148–150 °C; δ_P ($CDCl_3$): 33.48 (t, $^4J_{PF}$ = 6.9 Hz); δ_H ($CDCl_3$): 1.13 (3H, dd, $^3J_{PH}$ = 19.0 Hz, $^3J_{HH}$ = 7.1 Hz, N-CH–CH_3), 1.16 (3H, s, CH_2–C–CH_3), 1.32 (3H, s, CH_2–C–CH_3), 3.56 (1H, qd, $^2J_{PH}$ = 11.6 Hz, $^3J_{HH}$ = 7.0 Hz, N–C*H*-CH_3), 3.95 (1H, dd, $^3J_{PH}$ = 15.7 Hz, $^2J_{HH}$ = 11.4 Hz, O–CH_2), 4.02 (1H, dd, $^2J_{HH}$ = 11.4 Hz, $^3J_{PH}$ = 8.1 Hz, O–CH_2), 6.90–7.00 (1H, m, C*H*ar), 7.55–7.65 (2H, m, C*H*ar); δ_C ($CDCl_3$): 15.3 (s, P-CH-*C*H_3), 23.0 (s, CH_2–C-*C*H_3), 26.9 (s, CH_2–C-*C*H_3), 47.4 (d, $^3J_{PC}$ = 4.9 Hz, CH_2-*C*-CH_3), 50.8 (d, $^1J_{PC}$ = 104.7 Hz, P-*C*-CH_3), 51.5 (s, CH_3-*C*-CH_2), 76.1 (d, $^2J_{PC}$ = 7.3 Hz), 108.1 (t, $^2J_{CF}$ = 24.9 Hz, *C*Har), 115.0–116.0 (m, *C*Har), 131.7 (dt, $^1J_{PC}$ = 118.6 Hz, $^3J_{CF}$ = 7.3 Hz, *C*ar), 162.6 (ddd, $^1J_{CF}$ = 252.9 Hz, $^3J_{CF}$ = 19.8 Hz, $^3J_{PC}$ = 11.3 Hz, *C*ar-F); HRMS (FAB^+) MH^+ calcd $C_{12}H_{17}F_2NO_2P$: 276.0965; found 276.0965; and **5c** (*trans* form, 38%); mp = 134.5–135.5 °C; δ_P ($CDCl_3$): 29.12 (t, $^4J_{PF}$ = 6.9 Hz); δ_H ($CDCl_3$): 1.14 (3H, s, CH_2-CH–CH_3), 1.21 (3H, dd, $^3J_{PH}$ = 17.4 Hz, $^3J_{HH}$ = 7.1 Hz, N-CH–CH_3), 1.43 (3H, s, CH_2–C–CH_3), 3.27 (1H, qd, $^2J_{PH}$ = 4.9 Hz, $^3J_{HH}$ = 7.0 Hz, N–C*H*-CH_3), 3.95 (1H, dd, $^3J_{PH}$ = 16.4 Hz, $^2J_{HH}$ = 11.4 Hz, O–CH_2), 4.39 (1H, dd, $^2J_{HH}$ = 11.4 Hz, $^3J_{PH}$ = 2.5 Hz, O–CH_2), 7.00–7.10 (1H, m, C*H*ar), 7.30–7.40 (2H, m, C*H*ar); δ_C ($CDCl_3$): 14.8 (s, P-CH-*C*H_3), 22.3 (s, CH_2–C-*C*H_3), 26.3 (s, CH_2–C-*C*H_3), 50.8 (d, $^1J_{PC}$ = 92.9 Hz, P-*C*-CH_3), 50.5 (d, $^3J_{PC}$ = 4.4 Hz, CH_2-*C*-CH_3), 74.6 (d, $^2J_{PC}$ = 5.1 Hz, O-*C*H_2), 108.4 (t, $^2J_{CF}$ = 24.9 Hz, *C*Har), 114.0–115.0 (m, *C*Har), 132.7 (dt, $^1J_{PC}$ = 122.2 Hz, $^3J_{CF}$ = 7.0 Hz, *C*ar), 161.9 (ddd, $^1J_{CF}$ = 253.6 Hz, $^3J_{CF}$ = 20.1 Hz, $^3J_{PC}$ = 11.0 Hz, *C*ar-F); HRMS (FAB^+) MH^+ calcd $C_{12}H_{17}F_2NO_2P$: 276.0965; found 276.0960.

Epimerization process of oxazaphosphinane (5a–b)

Pathway A. 2-Phenyl- or 3-chlorophenyl-3,5,5-trimethyl-2-oxo-[1,4,2]-oxazaphosphinanes pure *cis* form (**8a–b**, 1 mmol, obtained according pathway A) was dissolved in an aqueous concentrated hydrochloric acid solution (35%, 12.5 mL), thus the mixture was heated for 3 days to 50 °C. After alkalinisation with sodium hydroxide (pH = 10), the mixture was extracted with ethyl acetate (3 × 30 mL). The organics layers were mixed, dried with sodium sulfate, filtered and evaporated to give **5a–b** respectively in 70% and 74% yields.

Pathway B. Applying previous to method A from a mixture of crude *cis* and *trans* **8b** and **5b** (~1/1) obtained from procedure described in pathway B, **5b** is recovered pure, 65%.

X-Ray experiments

Crystals structures were solved with the *ab initio* charge flipping program Superflip[24] and refined using non-linear least-squares implemented in CRYSTALS.[25]

Crystal data for **8b**: Formula = $C_{12}H_{17}Cl_1N_1O_2P_1$, T = 173 K, M_r = 273.70 gmol^{-1}, crystal size = 0.40 × 0.50 × 0.55 mm^3, Monoclinic, space group $P121/c1$, α = 8.11924(19), b = 10.0431(2), c = 16.0275(4) Å, α = 90°, β = 96.047(2)°, γ = 90°, V = 1299.65(5) Å^3, Z = 4, ρ_c = 1.399 g cm^{-3}, μ = 0.407 mm^{-1}, θ_{max} = 32.318, 20115 reflections measured, 4167 unique, 3540 with $I > 2\sigma(I)$, refined parameters = 154, $R_1(I > 2\sigma(I))$ = 0.0303, w$R_2(I > 2\sigma(I))$ = 0.0336 R_1(all data) = 0.0373, wR_2(all data) = 0.0336, $\Delta\rho$(min/max) = −0.37/0.38 eÅ^{-3}. Crystal data for **5a**: Formula = $C_{12}H_{18}N_1O_2P_1$, T = 173 K, M_r = 239.24 g mol^{-1}, crystal size = 0.10 × 0.20 × 0.25 mm^3, Monoclinic, space group $P121/c1$, a = 13.4636(10), b = 8.4643(4), c = 12.3030(9) Å, α = 90°, β = 113.455(9)°, γ = 90°, V = 1286.20(17) Å^3, Z = 4, ρ_c = 1.235 g cm^{-3}, μ = 0.200 mm^{-1}, θ_{max} = 32.445, 21989 reflections measured, 4232 unique, 1451 with $I > 2\sigma(I)$, refined parameters = 145, $R_1(I > 2\sigma(I))$ = 0.0266, w$R_2(I > 2\sigma(I))$ = 0.0278 R_1(all data) = 0.1011, wR_2(all data) = 0.0278, $\Delta\rho$(min/max) = −0.22/0.26 eÅ^{-3}. Crystal data for **5b**: Formula = $C_{12}H_{17}Cl_1N_1O_2P_1$, T = 173 K, M_r = 273.69 g mol^{-1}, crystal size = 0.20 × 0.22 × 0.25 mm^3, monoclinic, space group $P21/c$, a = 13.4738(6), b = 8.3372(4), c = 12.4129(6) Å, α = 90°, β = 92.615(4)°, γ = 90°, V = 1392.93(11) Å^3, Z = 4, r_{calcd} = 1.305 gcm^{-3}, μ = 0.379 mm^{-1}, θ_{max} = 28.998, 6357 reflections measured, 3150 unique, 2199 with

$I > 2\sigma(I)$, refined parameters = 154, $R_1(I > 2\sigma(I)) = 0.0432$, $wR_2(I > 2\sigma(I)) = 0.0508$ R_1(all data) = 0.0715, wR_2(all data) = 0.0508, $\Delta\rho$(min/max) = –0.34/0.56 eÅ^{-3}.

Forced swimming test

Each mouse was placed individually in a glass cylinder (Ø 12 cm, height 24 cm) filled with water at a height of 12 cm. Water temperature was maintained at 23 °C. Each animal was forced to swim for 15 min on the first day. It was then allowed to return to its home cage. On the second day, compounds were injected 30 min before the test. Each mouse was placed again into the water and forced to swim for 6 min. The session was recorded by a CCD camera connected to a computer and movements were analyzed using the Videotrack® software (Viewpoint, Champagne-au-Mont-d'Or, France). Two levels of pixel changes were analyzed to discriminate between immobility and struggling and struggling and swimming. Since only immobility is unequivocally regarded as a parameter of behavioral despair, other behaviors were not analyzed. Statistical data were expressed as mean ± S.E.M. and analyzed using a one-way ANOVA (F value), followed by a Dunnett's test for multiple comparisons. The level of significance was $p < 0.05$.

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