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New 1,8-naphthyridine and quinoline derivatives as CB_2 selective agonists

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Abstract—A series of new 1,8-naphthyridine and quinoline derivatives were synthesized and evaluated for their cannabinoid receptor affinity. In particular, compounds **2**, **5**, **11**, and **13** showed a high CB_2 affinity and CB_2 versus CB_1 selectivity, in agreement with molecular modeling studies. Furthermore, compound **2** also exhibited in vivo antinociceptive effects.

Recent data indicate that CB_2 cannabinoid receptors participate in the control of peripheral pain,[1] inflammation,[2] osteoporosis,[3] growth of malignant gliomas,[4] tumors of immune origin,[5] and immunological disorders such as multiple sclerosis.[6] Furthermore, CB_2 agents could be exploited for prevention of Alzheimer's disease pathology, given the presence of the CB_2 receptor in brain microglial cells,[7] and CB_2 receptor agonists might provide neuroprotection by blockade of microglial activation.[8] CB_2 receptor selective agonists may be the basis for developing new drugs for the treatment of amyotrophic lateral sclerosis.[9] Finally, it is becoming increasingly clear that selective agonism of CB_2 receptors may also constitute a novel strategy for treating chronic pain.[10] For example, the CB_2-selective compound, AM1241, has been shown to be anti-inflammatory, analgesic, and efficacious against inflammatory and neuropathic pain when administered either locally or systemically.[11]

We have previously reported the CB_1 and CB_2 receptor affinities of a series of 1,8-naphthyridin- and quinolin-4(1H)-on-3-carboxamide derivatives.[12,13]

These compounds generally exhibit a remarkable CB_2 affinity, with a K_i <20 nM, which was also accompanied by high selectivity for the CB_2 receptor. Moreover, [^{35}S]GTPγ binding assays and functional studies on human basophils indicated that the 1,8-naphthyridin-4(1*H*)-on-3-carboxamide derivatives behaved as CB_1 and CB_2 receptor agonists.[13] Our work has also suggested that arylalkyl and carboxycycloalkylamide substituents in positions 1 and 3 are necessary for a selective affinity for the CB_2 receptor.[14]

In the present study, following these suggestions, new 1,8-naphthyridine, quinoline, and tricyclic analogs were synthesized and tested on membranes prepared from HEK-293 cells expressing the human CB_1 and CB_2 receptors, to determine their affinities towards both CB subtypes. Furthermore, in order to obtain information about SAR of these compounds, we docked them into the three-dimensional model of CB receptors that were recently constructed.[14] Furthermore one of the 1,8-naphthyridine derivatives, which showed high CB_2 affinity with a good CB_2 versus CB_1 selectivity, was tested in vivo using the hot-plate test for antinociceptive activity.

The synthetic routes of target compounds are depicted in Schemes 1–4.[15] As reported in Scheme 1, the treatment of *N*-cyclohexyl-1-benzyl-7-chloro-1,8-naphthyri-

Keywords: Cannabinoid; CB_1 receptor; CB_2 receptor; 1,8-Naphthyridine; Quinoline.
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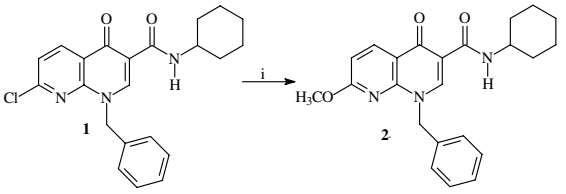
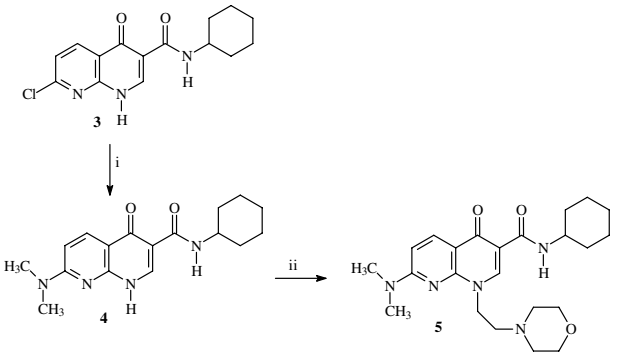
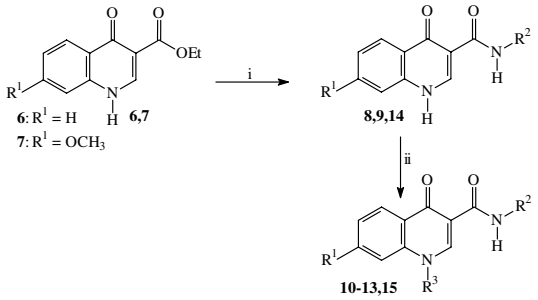


Scheme 1. Reagent and conditions: (i) MeONa, reflux, 6 h.



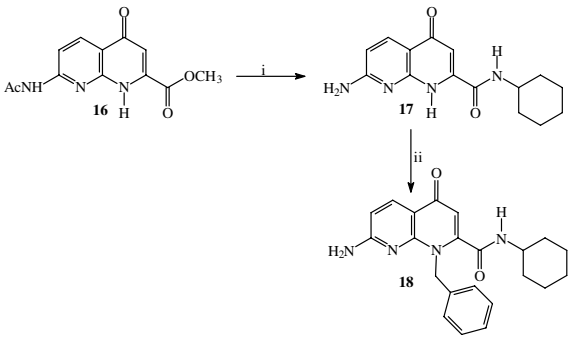
Scheme 2. Reagents and conditions: (i) dimethylamine, 120 °C, 24 h; (ii) DMF, NaH, 4-(2-chloroethyl)morpholine hydrochloride, 50 °C, 48 h.



Comp	R^1	R^2	R^3
8	OCH_3		--
9	H		--
14	Cl		--
10	OCH_3		$-CH_2-$
11	OCH_3		$-CH_2-CH_2-N$ O
12	H		$-CH_2-$
13	H		$-CH_2-CH_2-N$ O
15	Cl		$-CH_2-$

Scheme 3. Reagents and conditions: (i) cyclohexylamine or cycloheptylamine, 120 °C, 24 h; (ii) NaH, DMF, 4-(2-chloroethyl)morpholine hydrochloride or benzylchloride, 50 °C 24 h.

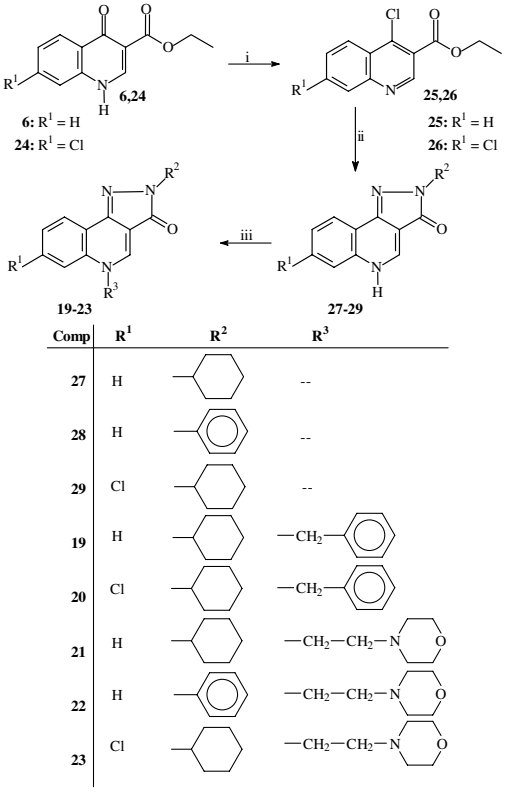
din-4(1H)-on-3-carboxamide (**1**)[13] with sodium methoxide in methanol under reflux for 6 h gave the 7-methoxy-1,8-naphthyridine **2** (Scheme 1) in 63% yield.



Scheme 4. Reagents and conditions: (i) cyclohexylamine, 120 °C, 24 h; (ii) benzylchloride, NaH, DMF, 50 °C, 24 h.

The 7-chloro-1,8-naphthyridine **3**,[12] by reaction with an excess of dimethylamine in a sealed tube at 120 °C for 24 h, gave *N*-cyclohexyl-7-(*N*,*N*-dimethylamine)-1,8-naphthyridin-4(1H)-on-3-carboxamide (**4**) (47% yield) which, in anhydrous DMF and NaH for 1 h at room temperature and then with 4-(2-chloroethyl)morpholine hydrochloride at 50 °C for 24 h, afforded the 1,8-naphthyridine derivative **5** (Scheme 2) in approximately 42% yield.

As reported in Scheme 3, the reaction of quinolin-4(1H)-on-3-carboxylic acid ethyl ester **6** or **7**[16] in a sealed tube with cyclohexylamine at 120 °C afforded the corresponding 3-carboxamide derivatives **8** (52% yield) or **9**



Comp	R^1	R^2	R^3
27	H		--
28	H		--
29	Cl		--
19	H		$-CH_2-$
20	Cl		$-CH_2-$
21	H		$-CH_2-CH_2-N$ O
22	H		$-CH_2-CH_2-N$ O
23	Cl		$-CH_2-CH_2-N$ O

Scheme 5. Reagents and conditions: (i) $POCl_3$, 120 °C, 15 min; (ii) R^2NHNH_2 150 °C, 13 h; (iii) benzylchloride or 4-(2-chloroethyl)morpholine hydrochloride, 24 h at rt or 50 °C or 70 °C.

(77% yield), respectively, which by treatment with NaH and then with benzylchloride or 4-(2-chloroethyl)morpholine hydrochloride gave the desired compounds **10**–**13** in 50–66% yields. In the same manner, treatment of *N*-cyclohexyl-7-chloroquinolin-4(1*H*)-on-3-carboxamide (**14**)[13] with NaH and then with benzylchloride afforded the quinolin-4(1*H*)-on-3-carboxamide derivative **15** (Scheme 3) in 60% yield.

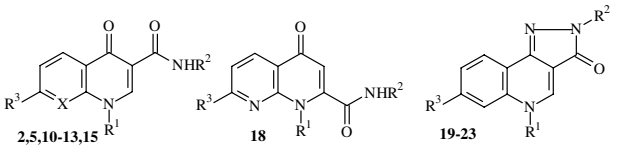
The 7-acetamido-1,8-naphthyridin-4(1*H*)-on-2-carboxylic acid methyl ester **16**[17] was heated at 120 °C in a sealed tube with cyclohexylamine for 24 h (Scheme 4). Under these conditions, the hydrolysis of the acetamido group also takes place, affording the 7-amino-2-carboxamide derivative **17** (30% yield). This compound, by reaction with benzylchloride under the same conditions described above, gave the 1,8-naphthyridine derivative **18** (Scheme 4) in very low yield (13%).

The synthesis of 3H-pyrazol-[4,3-*c*]-quinolines **19**–**23** is reported in Scheme 5. When the quinolin-4(1H)-on-3-carboxylic acid ethyl esters **6** and **24**[17] were heated with phosphoryl trichloride at 120 °C for 15 min the corresponding ethyl 4-chloro-3-quinolinecarboxylates **25**[18] (63% yield) and **26** (65% yield) were formed. These intermediates were subsequently converted into compounds **27**–**29** by reaction with appropriate hydrazines in xylene at 150 °C for 13 h (73–80% yields). The reaction of these with benzylchlorides or 4-(2-chloroethyl)-morpholine hydrochlorides under the same conditions described above gave the desired compounds **19**–**23** (30–60% yields).

The CB_1 and CB_2 receptor binding assay results for the new compounds are summarized in Table 1.[19,20] The K_i values of WIN-55,212-2, HU-210, AM630, JWH-133, ACEA, SR141716A and SR144528 are also included in the table as reference compounds for the CB_1 and CB_2 cannabinoid receptors.

The results indicate that the 1,8-naphthyridine and quinoline derivatives **2**, **5**, and **10**–**13** generally exhibit higher affinity for the CB_2 versus the CB_1 receptor. These data are in agreement with those previously reported for similar series of 1,8-naphthyridin- and quinolin-4(1H)-on-3-carboxamide derivatives[12,13] determined by measuring their ability to displace [^{3}H]CP-55,940 from its binding site in a membrane preparation from mouse brain (minus cerebellum) and mouse spleen homogenate, respectively. In particular, compounds **2** and **12** showed high CB_2 affinity with a K_i value of 11 and 6.4 nM, respectively. Moreover, the naphthyridine derivative **2** also possesses remarkable CB_2 receptor selectivity, with $K_i(CB_1)/K_i(CB_2) = 51$. Basing on our previous studies on

Table 1. Radioligand binding data of compounds **2**, **5**, **10**–**13**, **15**, **18**–**23**[a]



Compound	R^1	R^2	R^3	X	K_i (nM)		
					CB_1[b]	CB_2[c]	K_iCB_1/K_iCB_2
2	Benzyl	Cyclohexyl	OCH_3	N	560	11	51
5	Ethylmorph	Cyclohexyl	$N(CH_3)_2$	N	>5600	94	—
10	Benzyl	Cyclohexyl	OCH_3	C	170	79	2.1
11	Ethylmorph	Cyclohexyl	OCH_3	C	2920	79	37
12	Benzyl	Cycloheptyl	H	C	17	6.4	2.6
13	Ethylmorph	Cycloheptyl	H	C	290	28	10.3
15	Benzyl	Cyclohexyl	Cl	C	NT	NT	—
18	Benzyl	Cyclohexyl	NH_2	—	5600	7900	0.71
19	Benzyl	Cyclohexyl	H	—	620	790	0.78
20	Benzyl	Cyclohexyl	Cl	—	560	1640	0.34
21	Ethylmorph	Cyclohexyl	H	—	>5600	>7900	—
22	Ethylmorph	Phenyl	H	—	2800	2280	1.2
23	Ethylmorph	Cyclohexyl	Cl	—	NT	NT	—
WIN-55,212-2					21	2.1	10
HU-210					0.18	0.15	1.2
AM630					840	36	23
ACEA					5.3	95	0.06
JWH-130					680	3	227
SR141716A					16	1640	0.01
SR144528					>5000	5.4	—

NT = not tested as insoluble in the solvent normally used in binding assays.

[a] Data represent mean values for at least three separate experiments performed in duplicate and are expressed as K_i (nM), for CB_1 and CB_2 binding assays. Standard error of means (SEM) is not shown for the sake of clarity and was never higher than 5% of the means.

[b] Affinity of compounds for CB_1 receptor was evaluated using membranes from HEK-293 cells transfected and [^{3}H]CP55,940.

[c] Affinity of compounds for CB_2 receptor was evaluated using membranes from HEK-293 cells transfected and [^{3}H]CP-55,940.

1,8-naphthyridin- and quinolin-4(1H)-on-3-carboxamide derivatives, we hypothesize that these compounds (**2**, **5**, and **10**–**13**) behave as CB_2 agonists.[13]

The shifting of the carboxamide group from position 3 to position 2 of the heterocyclic nucleus together with the substitution of the methoxy group in position 7 with the amino group causes a drastic decrease of affinity toward both cannabinoid receptors, as confirmed by a comparison of compound **18** with **2**.

Finally, the tricyclic analogs **19**–**22**, characterized by the 2H-pyrazolo[4,3-*c*]quinolin-3(5H)-one central scaffold, showed a very low affinity toward both CB receptor subtypes.

The synthesized ligands were docked using AUTODOCK 3.0,[21] in the CB_1 and CB_2 receptor models prepared by molecular modeling.[14] Figure 1 shows the docking of compound **2** into both receptors; in the CB_1 receptor model, the naphthyridine and the cyclohexyl ring predominantly interact in the lipophilic pocket delimited by W5.43(279), T5.47(283), W6.48(356), L6.52(360), and V6.56(364), while the benzyl group is positioned in a secondary lipophilic pocket formed by L3.26(190), P4.60(251), and L4.61(252).

In the CB_2 receptor model, the benzyl ring of the ligand interacted similarly to the CB_1 receptor in the lipophilic pocket delimited by L3.27(108), P4.60(168) and L4.61(169) whereas the naphthyridine ring was rotated about 130° (counterclockwise sense from the extracellular point of view) and mainly interacted with W5.43(194), M6.55(265), and L6.60(269). Furthermore the methoxy substituent formed an H bond with the non-conserved S6.58(268) (substituted by D366 in the CB_1 receptor) and the cyclohexyl group, beyond the lipophilic interactions with T3.37(118), W5.43(194), and W6.48(258), feels the effect of a strong interaction with F5.46(197), which is a non-conserved residue (V282 in the CB_1). The interactions with S6.58(268) and F5.46(197), together with the different disposition of the naphthyridine ring, could be the reason for the CB_2 versus CB_1 selectivity of this ligand. Site-directed mutagenesis partially confirms our hypothesis, as mutations in the CB_2 subtype of F5.46(197) cause a substantial decrease of WIN-55,212-2 affinity.[22]

Compound **20** is the only one that highlights a CB_1 versus CB_2 selectivity profile. As shown in Figure A reported in the Supporting Information, compound **20** shows a disposition in the CB_1 binding site very similar to the one observed for compound **2**, with the same lipophilic interactions, in agreement with the very similar CB_1 affinity value. On the other hand, in the CB_2 binding site, the benzyl group was directed toward S6.58(268) and lost lipophilic interactions inside the pocket delimited by L3.27(108), P4.60(168) and L4.61(169), which explains its low CB_2 affinity.

Compound **2** which showed a high CB_2 affinity and selectivity (K_i = 11 nM, $K_i(CB_1)/K_i(CB_2)$ = 51) was tested to determine antinociceptive effects in the mouse

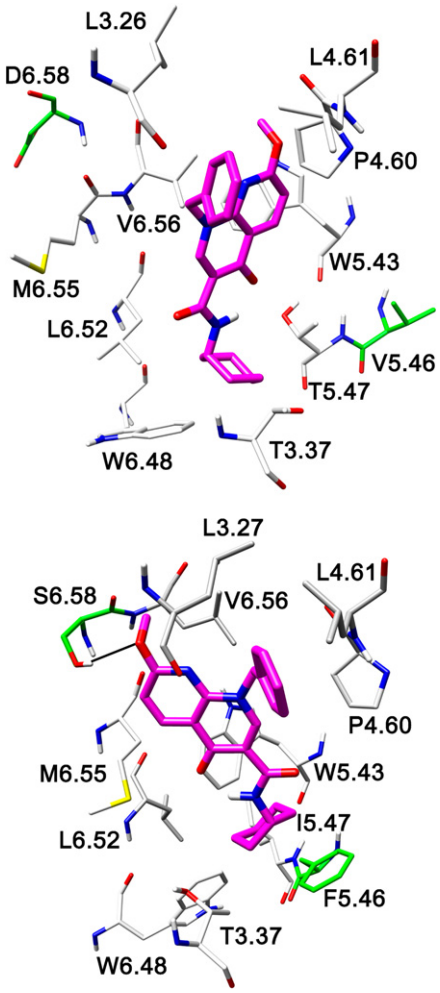


Figure 1. Compound **2** docked into CB_1 (up) and CB_2 (down) receptors, the non-conserved residues V/F5.46 and D/S6.58 and are colored green.

hot-plate test.[23,24] Furthermore, mouse motor coordination was evaluated in the rotarod test.[25,26] All experimental results are given as means ± SEM. An analysis of variance, ANOVA, followed by Fisher's protected least significant difference procedure for post hoc comparison, was used to verify statistically significant differences between two means of behavioral results. Data were analyzed with StatView software for the Macintosh (1992). *P* values less than 0.05 were considered statistically significant.

Compound **2** exhibited an antinociceptive effect at the dose of 50 mg kg^{-1} po in the mouse hot-plate test 15 and 30 min after administration (see Table 2). Under the same experimental conditions the doses of 10 and 30 mg kg^{-1} po of **2** were unable to increase pain threshold in a statistically significant manner. Treatment with AM630, a selective CB_2 antagonist,[27] at the dose of 0.6 mg kg^{-1} po partially reverted the antinociceptive effect of **2** in correspondence of its peak of efficacy. The dose of AM630 employed (0.6 mg kg^{-1} po) did not modify by itself mouse pain threshold in the presence of a thermal stimulus and represents the minimal dose able to reduce the antinociception induced by compound **2**. Moreover, this dose did not alter analgesia in-

Table 2. Effect of AM630 on antinociception induced by derivative **2** in the mouse hot-plate test

Treatment 1 po	Treatment 2 po	No. of mice	Licking latency (s)			
			Before pre treatment	After treatment		
				15 min	30 min	45 min
CMC	CMC	12	14.8 ± 0.6	15.5 ± 0.7	16.8 ± 0.6	14.7 ± 0.5
CMC	AM630 0.6 mg kg^{-1}	14	15.2 ± 0.4	16.6 ± 0.6	18.0 ± 0.4	16.3 ± 0.4
CMC	**2** 10 mg kg^{-1}	10	14.5 ± 0.8	17.1 ± 0.9	18.5 ± 1.0	16.7 ± 0.6
CMC	**2** 30 mg kg^{-1}	10	14.4 ± 0.6	16.6 ± 0.8	19.5 ± 1.0	17.4 ± 0.6
CMC	**2** 50 mg kg^{-1}	13	15.1 ± 0.5	19.2 ± 0.6 *	22.5 ± 0.9*	18.2 ± 0.7
2 50 mg kg^{-1}	AM630 0.6 mg kg^{-1}	10	16.2 ± 0.7	18.1 ± 0.7	19.2 ± 0.4^	17.6 ± 0.6

* $P < 0.05$ versus CMC-treated mice.
^ $P < 0.05$ versus 2-treated mice. AM-630 and 2 were administered simultaneously.

Table 3. Effect of 2 on motor coordination in the mouse rotarod test

Treatment po	No. of mice	No. of falls			
		Before treatment	After treatment		
			15 min	30 min	45 min
CMC	12	3.3 ± 0.2	0.8 ± 0.2	0.3 ± 0.2	0.2 ± 0.2
2 50 mg kg^{-1}	13	4.0 ± 0.7	0.7 ± 0.2	0.2 ± 0.2	0.2 ± 0.2

duced by the activation of opioid and GABAergic activation (data not shown) suggesting that dose is selective for CB neurotransmission.

However, AM630, at doses higher than the one mentioned above, could not be used since it caused an impairment of behavioral parameters (data not shown).

It is interesting to note that **2** at the antinociceptive dose did not modify mouse motor coordination evaluated in the rotarod test (see Table 3).

The lack of any impairment in the motor coordination of animals was demonstrated by the reduction of number of falls from the rotating rod before and 15, 30, 45, and 60 min after the beginning of the rotarod session.

In conclusion, the new 1,8-naphthyridine and quinoline derivatives synthesized and characterized confirmed the main suggested structural requisites for the CB_2 interaction. Moreover, compound **2** showed a high CB_2 affinity and CB_2 versus CB_1 selectivity and possessed antinociceptive effects, thus encouraging deeper studies on these classes of CB_2 agonists.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.09.089.

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19. For CB_1 and CB_2 receptor binding assays, the new compounds were tested using membranes from HEK-293 cells transfected with either the human CB_1 or CB_2 receptor and [^{3}H]-(−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)-phenyl]-*trans*-4-(3-hydroxy-propyl)-cyclohexanol ([^{3}H]CP-55,940) (K_d = 0.31 nM for CB_2 and 0.18 nM for

CB_1 receptors) as the high affinity ligand as described by the manufacturer (Perkin-Elmer, Italia).[19] Displacement curves were generated by incubating drugs with [^{3}H]CP-55,940 (0.084 for CB_2 and 0.14 nM for CB_1 binding assay). In all cases, K_i values were calculated by applying the Cheng–Prusoff equation to the IC_{50} values (obtained by GraphPad) for the displacement of the bound radioligand by increasing concentrations of the test compounds.

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24. Male Swiss albino mice (24–26 g) from Morini (San Polo d'Enza, Italy) were used. Ten mice were housed per cage. The cages were placed in the experimental room 24 h before the test for acclimatization. The animals were fed a standard laboratory diet and tap water ad libitum and kept at 22 ± 1 °C with a 12 h light/dark cycle, light at 7 a.m. All experiments were carried out in accordance with the NIH Guide for the Care and Use of Laboratory animals. All efforts were made to minimize animal suffering and to reduce the number of animals used. Mice were placed inside a stainless steel container, which was set thermostatically at 52.5 ± 0.1 °C in a precision water bath from KW Mechanical Workshop, Siena, Italy. Reaction times (s) were measured with a stopwatch before and 15, 30, 45, and 60 min after administration of analgesic drugs. The endpoint used was the licking of the fore or hind paws. Those mice scoring less than 12 and more than 18 s in the pretest were rejected (30%). An arbitrary cut-off time of 45 s was adopted to avoid injury. No sign of tissue injury was observed up to 45 s. Ten mice per group were tested.
25. The apparatus consisted of a base platform and a rotating rod with a diameter of 3 cm and a non-slippery surface. The rod was placed at a height of 15 cm from the base. The rod, 30 cm in length, was divided into five equal sections by six disks. Thus, up to five mice were tested simultaneously on the apparatus, with a rod-rotating speed of 16 rpm. The integrity of motor coordination was assessed on the basis of the number of falls from the rod in 30 s according to Vaught et al. Those mice scoring less than 3 and more than 6 falls in the pretest were rejected (20%). The performance time was measured before (pretest) and 15, 30, and 45 min after the beginning of the test. Ten mice per group were tested.
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