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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and analgesic activity of new pyridine-based heterocyclic derivatives

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Abstract A series of new heterocyclic derivatives having a pyridine nucleus were synthesized. 4-(5-(2-Chlorophenyl)-4H-1,2,4-triazol-3-yl)pyridine (**7c**) and 4-(5-(2-Nitrophenyl)-4H-1,2,4-triazol-3-yl)pyridine (**7d**) presented the best analgesic profie of this series in hot-plate, tail-flick, and formalin-induced licking tests, which was partially prevented by pretreatment with mecamylamine, a nicotinic receptor antagonist.

Keywords Pyridine · Nicotine · Epibatidine · Mecamylamine · Analgesic activity

Introduction

Pain is a typical sensory experience that may be described as the unpleasant awareness of a noxious stimulus or bodily harm. It is initiated by stimulation of nociceptors in the peripheral nervous system, or by damage to or malfunction of the peripheral or central nervous systems. The term analgesic means a drug that selectively relives pain by acting in the central nervous system or peripheral pain mechanisms, without significantly altering consciousness (Bennett and Villa, 2000). Though a host of popular analgesics are available, they suffer from side effects like dependence, tolerance, and gastrointestinal effects (Reisine and Pasternak, 1996; Insel, 1996). Hence, there is a need to explore new targets and agents acting on these targets. Neuronal nicotinic receptors have been implicated in a number of conditions like schizophrenia, autism, Alzheimer's disease, Parkinson's disease, dementia with Lewy bodies, and mood and anxiety disorders, besides pain (Whitehouse *et al*., 1988; Court *et al*., 2000; Perry *et al*., 2001; Lee *et al*., 2002; Pimlott *et al*., 2004; Bohr *et al*., 2005; Marutle *et al*., 2001; Newhouse *et al*., 2004; Ripoll *et al*., 2004). The literature reveals synthesis and analgesic activity of nicotine and epibatidine analogs acting through these receptors. Most of these analogs contain a pyridine nucleus (Fig. 1) (Bai *et al*., 1996; Corey *et al*., 1993; Holladay *et al*., 1998; Silva *et al*., 2002).

The reported analogs generally contain another heterocyclic ring attached at the 3rd position of pyridine ring. In this study, we report synthesis of some pyridine-based analogs substituted with different heterocyclic rings attached to the pyridine nucleus at the 3rd and 4th positions directly (**7**–**12**) or through a carbonyl linkage (**3**–**6**).

Chemistry

The target compounds, 5-methyl-1-(pyridin-4-yl carbonyl)-4-pyrazoline-3-(2H)-one (**3**) and 5-methyl-1-(pyridin-3-yl carbonyl)-4-pyrazoline-3-(2H)-one (**4**), were synthesized by reacting isoniazid and nicotinohydrazide, respectively, with ethylacetoacetate in the presence of acetic acid (Scheme 1). Similarly, 4-[(3,5-dimethyl-1H-pyrazol-1-yl)carbonyl]pyridine (**5**) and 3-[(3,5-dimethyl-1H-pyrazol-1-yl)carbonyl] pyridine (**6**) were synthesized by reacting isoniazid and nicotinohydrazide, respectively, with acetyl acetone in the presence of acetic acid (Scheme 1). Compounds 4-(5-substituted-4H-1,2,4-triazol-3-yl)pyridine **7**(**a**–**d**) and 3-(5-substituted-4H-1,2,4-triazol-3-yl)pyridine **8**(**a**–**d**) were synthesized by reacting isoniazid and nicotinohydrazide,

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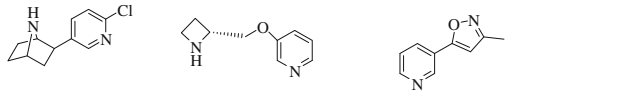
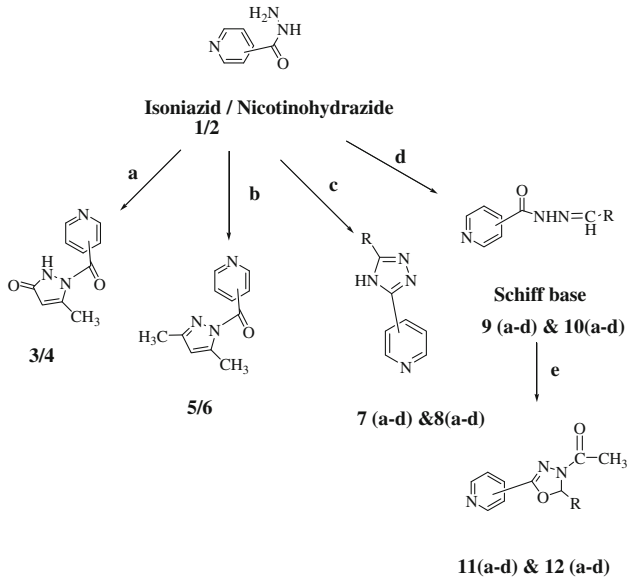


Epibatidine **ABT-594** **2-(3-methyl-5-isoxazolyl) pyridine**

Fig. 1 Epibatidine and epibatidine analogs contain a pyridine nucleus

respectively, with different aldyhydes in the presence of ammonium acetate (Scheme 1).

1-(2-Substituted-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2H)yl) ethanone **11**(**a**–**d**) and 1-(2-substituted-5-(pyridin-3-yl)-1,3,4-oxadiazol-3(2H)yl) ethanone **12**(**a**–**d**) were synthesized by reacting Schiff's base of isoniazid **9**(**a**–**d**) and nicotinohydrazide **10**(**a**–**d**), respectively, with acetic anhydride. Schiff's base **9**(**a**–**d**) and **10**(**a**–**d**) were synthesized by the addition of isoniazid and nicotinohydrazide, respectively, to aldehydes (Scheme 1).



Compounds	R
7a, 8a, 9a, 10a, 11a, 12a	-CH_3
7b, 8b, 9b, 10b, 11b, 12b	
7c, 8c, 9c, 10c, 11c, 12c	
7d, 8d, 9d, 10d, 11d, 12d	

Scheme 1 Synthesis of pyridine analogs. Reagents and conditions: (*a*) ethylacetoacetate, acetic acid, reflux—3 h; (*b*) acetylacetone, acetic acid, reflux—3 h; (*c*) substituted aldehyde, ammonium acetate, with stirring—24 h, and reflux—4 h; (*d*) substituted aldehyde, with reflux—3 h; (*e*) acetic anhydride, with reflux—1 h

Biology

Three acute pain models—tail-flick, hot-plate, and formalin-induced licking tests—were used to evaluate the synthesized compounds. The results are listed in Tables 1, 2, and 3. In the tail-flick test of D'Amour and Smith (1941), the tail is exposed to heat, and the amount of time taken for the animal to move (flick) its tail away from the heat is recorded. A control response (2–4 s) was determined for each mouse before treatment, and test latency was determined after drug administration. For the hot-plate test (Eddy and Leimbach, 1953; Atwell and Jacobson, 1978), mice were placed into a 10-cm-wide glass cylinder on a hot plate (Thermo just apparatus) maintained at 55.0°C. Two control latencies with at least 10 min apart were determined for each mouse. The normal latency (reaction time) was 8–10 s. The reaction time was scored when the animal jumped or licked its paws. The mice were tested 30 min after i.p. injections of the compounds (25 mg/kg) for the dose–response determination.

In the formalin-induced licking test (Shibata *et al.*, 1989) mice injected (i.p.) with test compounds (25 mg/kg) or vehicle (0.1% gum acacia solution). After 30 min, the mice were injected with 0.025 ml of 5% formalin into the dorsal portion of the front paw. Each individual mouse was placed into a clear plastic cage for observation. Pain responses were indicated by elevation or favoring of the paw, or excessive licking and biting of the paw. A biphasic response with an early (0–5 min) and a late (10–20 min) phase with high licking activity were observed.

Since several pyridine-based molecules have been found to produce analgesia through their action on neuronal nicotinic receptors, it was thought worthwhile to test the more active synthesized compounds for nAChR inhibition using mecamylamine antagonism test. Mecamylamine is a nAChR antagonist and thus is able to block analgesic action produced through activation of these receptors.

To evaluate whether the antinociceptive effects of synthesized compounds involved neuronal nicotinic receptors, separate groups of animals were pretreated with either vehicle or mecamylamine (2 mg/kg) (Rui *et al.*, 1996; Free *et al.*, 2006; Young and Shytle, 2001), and 20 min later, were treated with a second injection of either saline or the most effective synthesized compounds (25 mg/kg). After an additional 30 min, all the animals were tested for tail-flick test.

Comparisons between experimental groups were made using one-way analysis of variance (ANOVA) followed by

Table 1 Analgesic activity and mecamylamine antagonism of synthesized compounds in mice by tail-flick Test

Compound (25 mg/kg)	Latency time (s) mean (±SEM)	Latency time (s) mean (±SEM) (mecamylamine antagonism)	Compound (25 mg/kg)	Latency time (s) mean (±SEM)	Latency time (s) mean (±SEM) (mecamylamine antagonism)
Control	4.55 (0.09292)	4.55 (0.09292)	**8b**	4.73 (0.09292)	ND
Pentazocine[a]	12.84 (0.1877**)	ND	**8c**	5.53 (0.0881*)	ND
3	5.7 (0.2309**)	ND	**8d**	6.15 (0.0851*)	ND
4	5.26 (0.03)	ND	**11a**	11.03 (0.0656**)	5.14 (0.02186**)
5	6.40 (0.4064**)	ND	**11b**	6.03 (0.1764**)	ND
6	7.69 (0.1906**)	5.18 (0.0928**)	**11c**	6.03 (0.5457**)	ND
7a	7.62 (0.2709**)	5.22 (0.05044**)	**11d**	4.76 (0.1054**)	ND
7b	5.33 (0.1426)	ND	**12a**	8.95 (0.1906**)	5.14 (0.04041**)
7c	11.2 (0.3606**)	5.12 (0.01**)	**12b**	8.44 (0.084**)	5.13 (0.02333**)
7d	11.8 (0.2082**)	5.23 (0.07424**)	**12c**	5.16 (0.066)	ND
8a	7.99 (0.1054**)	5.17 (0.05364**)	**12d**	4.93 (0.1202)	ND

Results are expressed as means ± SEM and significant with * $P < 0.05$, ** $P < 0.01$. $N = 6$ mice per group

ND not done

[a] 17.5 mg/kg

Table 2 Analgesic activity of synthesized compounds in mice by hot-plate test

Compound (25 mg/kg)	Latency time (s) mean (±SEM)	Compound (25 mg/kg)	Latency time (s) mean (±SEM)
Control	7.26 (0.1457)	**8b**	7.50 (0.4508)
Pentazocine[a]	19.89 (0.1767**)	**8c**	8.55 (0.3044**)
3	7.51 (0.3592)	**8d**	8.47 (0.3716**)
4	7.54 (0.3460)	**11a**	12.93 (0.1079**)
5	8.11 (0.0152**)	**11b**	7.78 (0.2718)
6	8.30 (0.1650**)	**11c**	7.81 (0.1872)
7a	9.49 (0.3755**)	**11d**	7.48 (0.1054)
7b	7.81 (0.1682)	**12a**	10.03 (0.0680**)
7c	13.12 (0.1320**)	**12b**	10.03 (0.2203**)
7d	15.10 (0.4394**)	**12c**	7.77 (0.2868)
8a	9.65 (0.2203**)	**12d**	7.29 (0.1266)

Results are expressed as means ± SEM and significant with * $P < 0.05$, ** $P < 0.01$. $N = 6$ mice per group

[a] 17.5 mg/kg

Table 3 Analgesic activity of synthesized compounds in mice by formalin-induced licking test

Compound (25 mg/kg)	Number of paw licking (0–5 min) mean (±SEM)	Number of paw licking (10–20 min) mean (±SEM)	Compound (25 mg/kg)	Number of paw licking (0–5 min) mean (±SEM)	Number of paw licking (10–20 min) mean (±SEM)
Control	11.66 (0.8819)	11.33 (0.8819)	**8b**	7.66 (0.333*)	6 (1.155**)
Pentazocine[a]	4.33 (0.333**)	3.33 (0.333**)	**8c**	8.33 (1.202)	7 (0.5774**)
3	10.33 (0.666)	4.33 (0.333**)	**8d**	9.33 (1.453)	6.66 (0.333**)
4	6.33 (0.8819**)	5.33 (0.333**)	**11a**	5.66 (0.333**)	3.66 (0.333**)
5	7.66 (0.333*)	4.66 (0.333**)	**11b**	7.33 (0.8819**)	4.33 (0.881**)
6	8.33 (0.666)	8 (1.00**)	**11c**	8.66 (0.8819)	5 (0.577**)
7a	6.66 (0.333**)	4 (0.577**)	**11d**	10.66 (1.202)	4.33 (0.666**)
7b	11 (0.5774)	6 (0.5774**)	**12a**	6.33 (0.333**)	5 (1.00**)
7c	5.33 (0.8819**)	4.33 (0.333**)	**12b**	8.66 (1.453)	7.66 (0.333**)
7d	5 (0.5774**)	4.33 (0.333**)	**12c**	10.33 (0.8819)	7.33 (0.333**)
8a	6.66 (0.333**)	5.66 (0.666**)	**12d**	9.66 (0.666)	8 (0.5774**)

Results are expressed as means ± SEM and significant with * $P < 0.05$, ** $P < 0.01$. $N = 6$ mice per group

[a] 17.5 mg/kg

Dunnet's multiple comparison test (dose–response and time course studies). All the data were analyzed using Graph Pad Instat. Statistical significance was accepted at a value of $P < 0.05$.

Results and discussion

The desired compounds were synthesized using very simple synthetic procedures outlined in Scheme 1. All the compounds were characterized by IR and ^{1}H NMR spectroscopy. These compounds when evaluated for analgesic action were found to elevate the latency time in the tail-flick and hot-plate tests. Significant reduction in the number of paw licking was observed in formalin-induced licking test.

IR (KBr) spectra of compounds **3–6** reveal a characteristic aromatic stretch between 3,034 and 3,100 cm^{-1} and sharp carbonyl stretching vibration between 1,681 and 1,690 cm^{-1}. The stretching vibrations for C=N group of pyrazolone are seen at 1,501–1,576 cm^{-1}. IR (KBr) spectra of compounds **7**(**a–d**) and **8**(**a–d**) reveal a characteristic NH stretching vibration for the triazole at 3,400–3,500 cm^{-1}. The IR spectra of compounds **11**(**a–d**) and **12**(**a–d**) show stretching vibrations for C=O group between 1,681 and 1,700 cm^{-1}. Also the disappearance of NH stretching vibration for intermediate Schiff's bases [**9**(**a–d**) **and 10**(**a–d**)] at 3,400–3,500 cm^{-1}, confirms the formation of 1,3,4-oxadiazoline ring.

The ^{1}H NMR spectra of 4-pyrazoline-3-(2H)-one derivatives (**3**, **4**) were recorded in DMSO-d_6. The pyridine protons showed peaks between 7.57–7.58 and 8.23–8.74 ppm as doublets. Pyrazoline NH appeared as singlet at 10.80 ppm. Pyrazoline 4-H displayed a peak at 5.81 ppm as singlet. Methyl protons of pyrazoline exhibited a singlet at 2.35 ppm. ^{1}H NMR spectra of pyrazole derivatives (**5**, **6**) were recorded in DMSO-d_6. The pyridine protons appeared as doublets between 7.57–7.58 and 8.23–8.27 ppm. Pyrazole 4-H showed a peak at 5.81 ppm as singlet. Pyrazole 3-CH_3 and 5-CH_3 showed peaks at 2.47 and 2.35 ppm, respectively, as singlet. ^{1}H NMR spectra of triazole derivatives [**7**(**a–d**) and **8**(**a–d**)] were recorded in DMSO-d_6. The pyridine protons showed peaks between 7.79–7.82 and 8.44–8.74 ppm as doublets in **7**(**a–d**). While in compounds **8**(**a–d**), the pyridine protons appeared as triplets at 7.71–8.09 ppm, as doublets at 8.14–8.75 and 8.24–9.24 ppm, and as singlet at 8.82–9.24 ppm. The aromatic protons appeared at 7.68–7.79 ppm as multiplet and at 8.14–8.24 ppm as doublet. Triazole NH showed peaks between 10.92 and 12.27 ppm. ^{1}H NMR spectra of all the oxadiazoline derivatives [**11**(**a–d**) and **12**(**a–d**)] were recorded in $CDCl_3$. The pyridine protons showed peaks between 8.10 and 8.20 ppm as triplets, at 7.91–8.70 ppm as doublets, and at 9.10–9.46 ppm as singlets. The aromatic protons displayed peaks at 7.49–7.89 ppm as multiplets. Oxadiazoline 5-H appeared between 6.20 and 6.60 ppm.

The analgesic activity of synthesized compounds was evaluated using tail-flick test, hot-plate test, and formalin-induced test. The data obtained are presented in Tables 1, 2, and 3, respectively, and expressed as mean ± SEM.

In tail-flick test, each group was injected with test drug 25 mg/kg (i.p.) or vehicle (0.1% gum acacia solution) (i.p.). Pentazocine 17.5 mg/kg (i.p.) was used as reference. As shown in Fig. 2, a single i.p. administration of the target compounds **6**, **7a**, **7c**, **7d**, **8a**, **11a**, **12a, and 12b** resulted in a remarkable analgesic effect.

In hot-plate test, each group was injected with test drug 25 mg/kg (i.p.) or vehicle (0.1% gum acacia solution) (i.p.). Pentazocine 17.5 mg/kg (i.p.) was used as reference. As evident in Fig. 3, compounds **6**, **7a**, **7c**, **7d**, **8a**, **11a**, and **12a** have displayed remarkable analgesic effects.

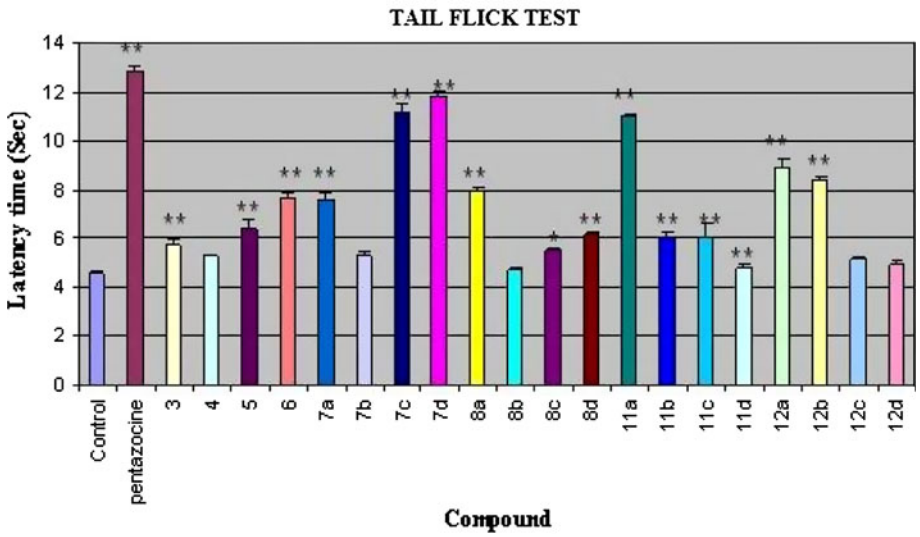


Fig. 2 Effect of i.p. administration of synthesized derivatives (25 mg/kg) during the course of the latency times in the tail-flick test in mice. Results are expressed as means ± SEM and significant with * $P < 0.05$, ** $P < 0.01$. $N = 6$ mice per group

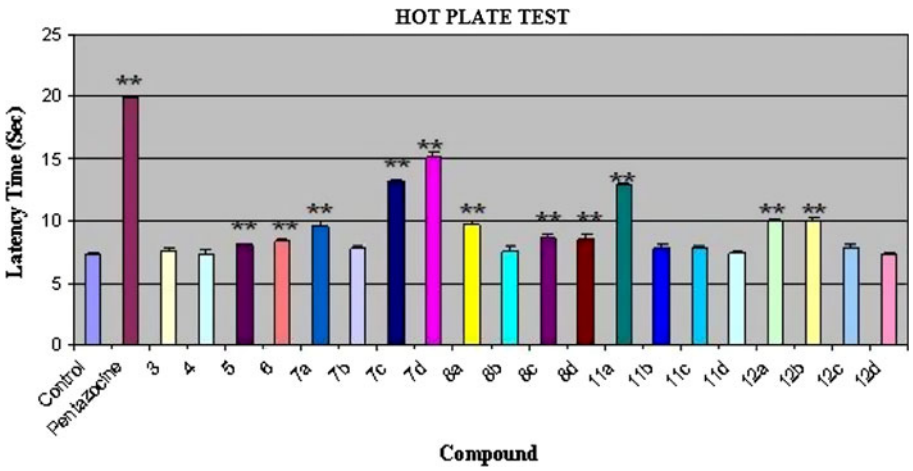


Fig. 3 Effect of i.p. administration of synthesized derivatives (25 mg/kg) during the course of the latency times in the hot-plate test in mice. Results are expressed as means ± SEM and significant with * $P < 0.05$, ** $P < 0.01$. $N = 6$ mice per group

In formalin-induced test. each group was injected with test drug 25 mg/kg (i.p.) or vehicle (0.1% gum acacia solution) (i.p.). Pentazocine 17.5 mg/kg (i.p.) was used as reference. After 30 min, each group was injected with 0.025 ml of 5% formalin into the dorsal portion of the front paw. Pain responses were indicated by elevation or favoring of the paw or excessive licking and biting of the paw. A biphasic response with an early (0–5 min) and a late (10–20 min) phase with high licking activity was observed. A single i.p. administration of the compounds **6**, **7a**, **7c**, **7d**, **8a**, **11a**, **12a, and 12b** resulted in a remarkable analgesic effect (Fig. 4).

Mecamylamine is a nAChR antagonist and thus is able to block analgesic action produced through activation of these receptors.

To evaluate whether the analgesic effects of synthesized compounds involved neuronal nicotinic receptors, separate groups of animals were pretreated with either vehicle or mecamylamine (2 mg/kg) (Rui *et al.*, 1996; Free *et al.*, 2006), and 20 min later, they were treated with a second injection of either saline or synthesized compounds **6**, **7a**, **7c**, **7d**, **8a**, **11a**, **12a**, and **12b** (25 mg/kg). After a 30 min, all the animals were tested for tail-flick test. As shown in Fig. 5, the analgesic effects of synthesized compounds **6**, **7a**, **7c**, **7d**, **8a**, **11a**, **12a**, and **12b** were reduced by mecamylamine.

Based on the activity data (Tables 1, 2, 3), the following observations were made:

(1) Compounds having a 5-membered heterocyclic ring containing three heteroatoms (**7a–d**, **8a–d**, **11a–d**, and **12a–d**) were more active than those containing ring with only two heteroatoms (**3–6**).
(2) 5-Substituted 1, 2, 4-triazol-3-yl at the 4th position of pyridine ring (**7a–d**) was found to enhance the analgesic activity as compared to when it is attached to the 3rd position of pyridine ring. Electron-withdrawing groups, like chloro and nitro, when substituted at ortho position of phenyl ring in triazole derivatives (**7c**, **7d**) increased the activity.
(3) Similarly, 2-substituted 1,3,4-oxadiazoline at the 4th position of pyridine ring (**11a**) possessed better analgesic activity as compared to when it is attached to the 3rd position of pyridine ring (**12a**).
(4) Methyl substitution on oxadiazoline ring (**11a**, **12a**) favors activity as compared with the substituted and unsubstituted phenyl rings (**11b**, **11c**, **11d**, **12b**, **12c**, and **12d**).

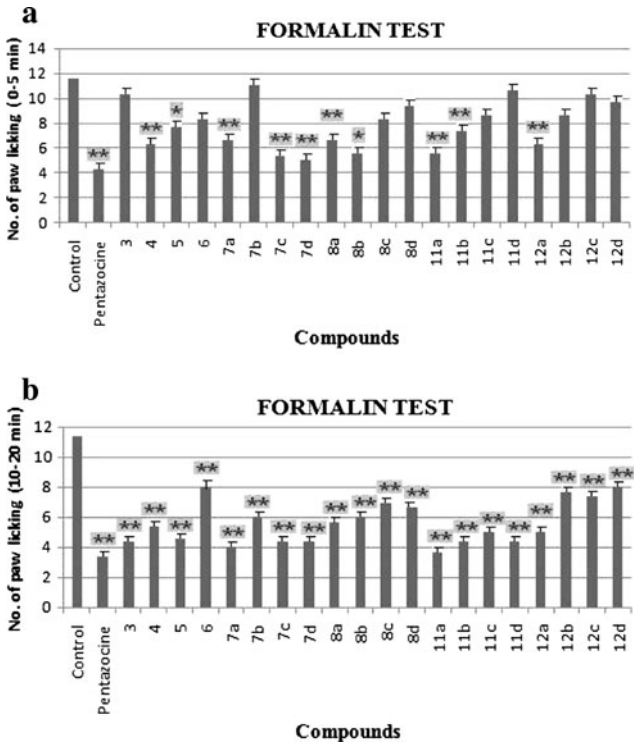


Fig. 4 Effect of i.p. administration of synthesized derivatives (25 mg/kg) on the course of the latency times in the formalin test in mice (**a**) 0–5 min (**b**) 10–20 min. Results are expressed as means ± SEM and significant with * $P < 0.05$, ** $P < 0.01$. $N = 6$ mice per group

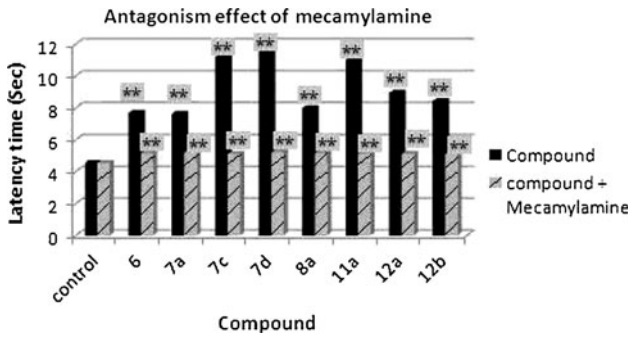


Fig. 5 Effect of mecamylamine (2 mg/kg) on synthesized derivative's elicited analgesia in mice. Results are expressed as means ± SEM and significant with * $P < 0.05$, ** $P < 0.01$. $N = 6$ mice per group. $C + M$ compound + mecamylamine, C compound

The compound **6**, **7a**, **7c**, **7d**, **8a**, **11a**, **12a, and 12b** (25 mg/kg, i.p.) were found to be the most active in all the tests. Moreover, the analgesic effect of these compounds was reduced by mecamylamine (Fig. 5) suggesting the possible involvement of neuronal AChRs in producing analgesic effects.

Conclusion

To conclude, results of this study suggest that the synthesized compounds **6**, **7a**, **7c**, **7d**, **8a**, **11a**, **12a**, and **12b** displayed the best analgesic profile of this series. Moreover, the analgesic effects of these compounds were greatly reduced by mecamylamine, the noncompetitive neuronal nicotinic acetylcholine receptor antagonist, suggesting the involvement of neuronal AChRs in producing analgesic effects besides any other mechanism.

Experimental

All the chemicals used in the synthesis were of laboratory grade. The melting points were determined in open capillary on Veego (model:-VMP-D) electronic apparatus and are uncorrected. The IR spectra of the synthesized compounds were recorded on Shimadzu 8400-S FT-IR Spectrophotometer using potassium bromide. The ^{1}H NMR were recorded in $CDCl_3$ and DMSO-d_6 using NMR Varian-Mercury 300 MHz spectrometer and chemical shifts are given in parts per millions, downfield from tetramethylsilane (TMS) as an internal standard. The reactions were monitored by pre-coated aluminum silica gel 60 F254 thin layer plates procured from Merck (Germany), using toluene–methanol, chloroform–ethyl acetate, and dioxane as the solvent systems, and the spots were visualized by exposure to iodine vapors.

5-Methyl-1-(pyridin-4-yl carbonyl)-4-pyrazoline-3-(2H)-one (**3**) and 5-methyl-1-(pyridin-3-yl carbonyl)-4-pyrazoline-3-(2H)-one (**4**)

Isoniazid (**1**) or nicotinohydrazide (**2**) 2.74 g (0.02 mol) and 2.60 ml (0.02 mol) ethylacetoacetate were dissolved in 30 ml ethanol taken in a 100-ml round-bottomed flask and refluxed for 3 h. To this solution was added 1–2 ml of acetic acid and refluxed again for 1 h. It was then poured into ice water. The separated solid was filtered, washed with water, and dried in air. The crude product was recrystallized from ethanol to yield pure product.

5-Methyl-1-(pyridin-4-yl carbonyl)-4-pyrazoline-3-(2H)-one (**3**)

Yield: 83%; orange, solid; mp 256–258°C.

IR (KBr, cm^{-1}): 1570.11 (C=N), 1593.25 (C=C), 1681.98 (C=O), 2980.12 (Me-CH), 3034.16, 3066.35 (Ar–CH), 3462.31 (NH).

^{1}H NMR (DMSO-d_6, δ ppm): 2.35 (s, 3H, CH_3), 5.81 (s, 1H, CH), 7.56 (d, 2H, $J = 5.6$, Py), 8.25 (d, 2H, $J = 5.7$, Py), 10.80 (s, 1H, NH).

5-Methyl-1-(pyridin-3-yl carbonyl)-4-pyrazoline-3-(2H)-one (**4**)

Yield: 73%; orange, solid; mp: 236–238°C.

IR (KBr, cm^{-1}): 1572.04 (C=N), 1597.11 (C=C), 1681.98 (C=O), 2956.97 (Me–CH), 3005.20, 3194.23 (Ar–CH), 3387.11 (NH).

^{1}H NMR (DMSO-d_6, δ ppm): 2.35 (s, 3H, CH_3), 5.81 (s, 1H, CH), 7.56 (t, 1H, $J = 7.4$, Py), 8.24 (d, 1H, $J = 5.6$, Py), 8.77 (d, 1H, $J = 5.6$, Py), 9.0 (s, 1H, Py), 10.80 (s, 1H, NH).

4-[(3,5-dimethyl-1H-pyrazol-1yl)carbonyl]pyridine (**5**) and 3-[(3,5-dimethyl-1H-pyrazol-1-yl)carbonyl]pyridine (**6**)

Isoniazid (**1**) or nicotinohydrazide (**2**) 2.74 g (0.02 mol) and 2.60 ml (0.02 mol) acetyl acetone were dissolved in 30 ml ethanol taken in a 100-ml round-bottomed flask and refluxed for 3 h. To this solution was added 1–2 ml of acetic acid and refluxed again for 1 h. The resulting solution was poured into ice water. The separated solid was filtered, washed with water, and dried in air. The crude product was recrystallized from ethanol to yield pure product.

4-[(3,5-dimethyl-1H-pyrazol-1-yl)carbonyl]pyridine (**5**)

Yield: 82%; white, solid; mp 234–236°C.

IR (KBr, cm^{-1}): 1570.11 (C=N), 1593.25 (C=C), 1698.43 (C=O), 2856.63 (Me–CH), 3034.13, 3074.63 (Ar–CH).

^{1}H NMR (DMSO-d_6, δ ppm): 2.36 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 5.82 (s, 1H, CH), 7.56 (d, 2H, $J = 5.6$, Py), 8.24 (d, 2H, $J = 5.6$, Py)

3-[(3,5-Dimethyl-1H-pyrazol-1-yl)carbonyl] pyridine (**6**)

Yield: 72%; brown, solid; mp 230–232°C.

IR (KBr, cm^{-1}): 1588.54 (C=N), 1593.25 (C=C), 1674.27 (C=O), 2846.53 (Me–CH), 3059.20, 3186.51 (Ar–CH).

^{1}H NMR (DMSO-d_6, δ ppm): 2.35 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 5.89 (s, 1H, CH), 7.56 (t, 1H, $J = 7.3$, Py), 8.25 (d, 1H, $J = 5.4$, Py), 8.77 (d, 1H, $J = 5.6$, Py), 9.05 (s, 1H, Py).

General procedure for synthesis of 4-(5-substituted-4H-1,2,4-triazol-3-yl)pyridine **7**(**a**–**d**) and 3-(5-substituted-4H-1,2,4-triazol-3-yl)pyridine **8**(**a**–**d**)

Equimolar quantities (0.01 mol) of isoniazid (**1**) or nicotinohydrazide (**2**), ammonium acetate, and the appropriate aldehyde were added to 15 ml acetic acid in 100-ml iodine flask and stirred for 24 h. The solution was then neutralized with liquid ammonia, and the resulting mixture was refluxed for 4 h. The solid separated was filtered, washed with water, and dried in air. The crude product was recrystallized from ethanol to yield pure product.

4-(5-Methyl-4H-1,2,4-triazol-3-yl)pyridine (**7a**)

Yield: 70%; brown, solid; mp 245–247°C.

IR (KBr, cm^{-1}): 1558.54 (C=N), 1606.76 (C=C), 2924.18 (Me–CH), 3064.58 (Ar–CH), 3431.48 (NH).

^{1}H NMR (DMSO-d_6, δ ppm): 2.49 (s, 3H, CH_3), 7.78 (d, 2H, $J = 5.3$, Py), 8.77 (d, 2H, $J = 5.6$, Py), 10.92 (s, 1H, NH).

4-(5-Phenyl-4H-1,2,4-triazol-3-yl)pyridine (**7b**)

Yield: 68%; yellow, solid; mp 241–242°C.

IR (KBr, cm^{-1}): 1566.25 (C=N), 1588.02 (C=C), 3026.11 (Ar–CH), 3450.10 (NH).

^{1}H NMR (DMSO-d_6, δ ppm): 7.46 (d, 2H, $J = 7.2$, Ar), 7.79 (t, 2H, $J = 8.6$, Ar), 7.81 (d, 2H, $J = 5.5$, Py), 8.44 (d, 1H, $J = 7.4$, Ar), 8.77 (d, 2H, $J = 5.6$, Py), 12.05 (s, 1H, NH).

4-(5-(2-Chlorophenyl)-4H-1,2,4-triazol-3-yl) pyridine (**7c**)

Yield: 71%; buff, solid; mp 215–217°C.

IR (KBr, cm^{-1}): 750.33 (C–Cl), 1570.11 (C=N), 3034.16, 3066.35 (Ar–CH), 3462.31 (NH).

^{1}H NMR (DMSO-d_6, δ ppm): 7.48 (d, 2H, $J = 7.5$, Ar), 7.72–7.78 (m, 2H, Ar), 8.40 (d, 2H, $J = 5.4$, Py), 8.73 (d, 2H, $J = 5.6$, Py),12.08 (s, 1H, NH).

4-(5-(2-Nitrophenyl)-4H-1,2,4-triazol-3-yl) pyridine (**7d**)

Yield: 66%; yellow, solid; mp 225–227°C.

IR (KBr, cm^{-1}): 1354.07, 1525.74 (C–NO_2), 1560.82 (C=N), 1600.57 (C=C), 3059.20, 3062.35 (Ar–CH), 3460.31 (NH).

^{1}H NMR (DMSO-d_6, δ ppm): 7.68–7.79 (m, 2H, Ar), 8.14 (d, 1H, $J = 7.4$, Ar), 8.24 (d, 1H, $J = 7.6$, Ar), 8.51 (d, 2H, $J = 5.4$, Py), 8.74 (d, 2H, $J = 5.5$, Py), 12.27 (s, 1H, NH).

3-(5-Methyl-4H-1,2,4-triazol-3-yl)pyridine (**8a**)

Yield: 60%; brown, solid; mp 152–154°C.

IR (KBr, cm^{-1}): 1554.56 (C=N), 1591.33 (C=C), 2872.18 (Me–CH), 3057.27–3070.78 (Ar–CH), 3392.50 (NH).

^{1}H NMR (DMSO-d_6, δ ppm): 2.49 (s, 3H, CH_3), 7.75 (t, 1H, $J = 7.2$, Py), 8.14 (d, 1H, $J = 5.6$, Py), 8.24 (d, 1H, $J = 5.6$, Py), 8.82 (s, 1H, Py), 12.27 (s, 1H, NH).

3-(5-Phenyl-4H-1,2,4-triazol-3-yl)pyridine (**8b**)

Yield: 68%; yellow, solid; mp 130–132°C.

IR (KBr, cm^{-1}): 1566.02 (C=N), 1596.25 (C=C), 3026.11 (Ar–CH), 3450.10 (NH).

^{1}H NMR (DMSO-d_6, δ ppm): 7.29–7.70 (m, 3H, Ar), 7.89 (t, 1H, $J = 7.2$, Py), 8.57 (d, 2H, $J = 7.2$, Ar), 8.75 (d, 1H, $J = 5.4$, Py), 9.24 (s, 1H, Py), 9.95 (d, 1H, $J = 5.6$, Py), 11.25 (s, 1H, NH).

3-(5-(2-Chlorophenyl)-4H-1,2,4-triazol-3-yl) pyridine (**8c**)

Yield: 73%; yellow, solid; mp 138–140°C.

IR (KBr, cm^{-1}): 761.91 (C–Cl), 1553.24 (C=N), 1593.25 (C=C), 3099.20, 3186.51 (Ar–CH), 3512.31 (NH).

^{1}H NMR (DMSO-d_6, δ ppm): 7.23–7.63 (m, 4H, Ar), 7.99 (t, 1H, $J = 7.1$, Py), 8.46 (d, 1H, $J = 5.6$, Py), 8.72 (d, 1H, $J = 5.6$, Py), 9.04 (s, 1H, Py), 12.17 (s, 1H, NH).

4-(5-(2-Nitrophenyl)-4H-1,2,4-triazol-3-yl) pyridine (**8d**)

Yield: 76%; yellow, solid; mp 145–147°C.

IR (KBr, cm^{-1}): 1355.43, 1524.28 (C–NO_2), 1554.64 (C=N), 1593.25 (C=C), 3099.20, 3186.51 (Ar–CH), 3512.31 (NH).

^{1}H NMR (DMSO-d_6, δ ppm): 7.56–7.73 (m, 4H, Ar), 8.05 (t, 1H, $J = 7.2$, Py), 8.23 (d, 1H, $J = 5.6$, Py), 8.78 (d, 1H, $J = 5.6$, Py), 9.00 (s, 1H, Py), 12.40 (s, 1H, NH).

Synthesis of target compounds

1-(2-substituted-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2H)yl)-ethanone **11**(**a**–**d**) and 1-(2-substituted-5-(pyridin-3-yl)-1,3,4-oxadiazol-3(2H)yl) ethanone **12**(**a**–**d**)

General procedure for synthesis of intermediate (Schiff's base) ***9(a–d) and 10(a–d)***

Isoniazid (**1**) or nicotinohydrazide (**2**) 1.37 g (0.01 mol) and (0.01 mol) of aldehyde were dissolved in 20 ml ethanol and refluxed for 3 h in a 100-ml round bottomed flask. The mixture was poured on to crushed ice, and the solid separated was washed with ethanol and dried in air. The crude product was recrystallized from ethanol to yield pure product.

N′-[ethylidene] pyridine-4-carbohydrazide (**9a**)

Yield: 81%; yellow, solid; mp 200–202°C.

IR (KBr, cm^{-1}): 1550.42 (C=N), 1604.23 (C=C), 1684.31 (C=O), 2903.13, 2935.43 (CH_3–CH), 3063.06, 3182.65 (Ar–CH), 3432.40 (NH).

N′-[phenylmethylidine] pyridine-4-carbohydrazide (**9b**)

Yield: 83%; yellow, solid; mp 203–205°C.

IR (KBr, cm^{-1}): 1581.93 (C=N), 1602.09 (C=C), 1696.49 (C=O), 2903.13, 3001.31 (Ar–CH), 3460.41 (NH).

N′-[(2-chlorophenyl) methylidine] pyridine-4-carbohydrazide (**9c**)

Yield: 82%; yellow, solid; mp 202–204°C.

IR (KBr, cm^{-1}): 761.91 (C–Cl), 1570.10 (C=N), 1593.25 (C=C), 3099.20, 3186.51 (Ar–CH), 3512.31 (NH).

^{1}H NMR (DMSO-d_6, δ ppm): 7.50–7.74 (m, 4H, Ar), 7.78 (d, 2H, $J = 5.5$, Py), 8.74 (d, 2H, $J = 5.6$, Py), 8.40 (s, 1H, CH), 12.08 (s, 1H, NH).

N′-[(2-nitrophenyl) methylidine] pyridine-4-carbohydrazide (**9d**)

Yield: 82%; yellow, solid; mp 203–205°C.

IR (KBr, cm^{-1}): 1350.07, 1522.58 (C–NO_2), 1569.33 (C=N), 1591.68 (C=C), 1684.19 (C=O), 3037.99, 3060.07 (Ar–CH), 3439.19 (NH).

N′-[ethylidene] pyridine-3-carbohydrazide (**10a**)

Yield: 73%; yellow, solid; mp 138–140°C.

IR (KBr, cm^{-1}): 1556.51 (C=N), 1596.61 (C=C), 1681.58 (C=O), 2963.43 (Me–CH), 3064.99, 3188.03 (Ar–CH), 3431.48 (NH).

N′-[phenylmethylidine] pyridine-3-carbohydrazide (**10b**)

Yield: 73%; yellow, solid; mp 130–132°C.

IR (KBr, cm^{-1}): 1581.37 (C=N), 1602.13 (C=C), 1688.79 (C=O), 2903.13, 3001.31 (Ar–CH), 3460.41 (NH).

N′-[(2-chlorophenyl) methylidine] pyridine-3-carbohydrazide (**10c**)

Yield: 75%; yellow, solid; mp 148–150°C.

IR (KBr, cm^{-1}): 750.12 (C–Cl), 1570.10 (C=N), 1593.25 (C=C), 1678.39 (C=O), 3023.65, 3034.13 (Ar–CH), 3466.20 (NH).

^{1}H NMR (DMSO-d_6, δ ppm): 7.53–7.75 (m, 4H, Ar), 8.05 (t, 1H, $J = 7.1$, Py), 8.23 (d, 1H, $J = 5.5$, Py), 8.44 (s, 1H, CH), 8.78 (d, 1H, $J = 5.6$, Py), 9.00 (s, 1H, Py), 12.40 (s, 1H, NH).

N′-[(2-nitrophenyl) methylidine] pyridine-3-carbohydrazide (**10d**)

Yield: 77%; yellow, solid; mp 142–144°C.

IR (KBr, cm^{-1}): 1342.50, 1519.96 (C–NO_2), 1552.75 (C=N), 1600.06 (C=C), 1681.12 (C=O), 3037.99, 3074.63 (Ar–CH), 3432.19 (NH).

General procedure for synthesis of target compound **11**(**a**–**d**) **and 12**(**a**–**d**)

Appropriate Schiff base (0.002 mol) was dissolved in 0.1 mol of acetic anhydride, and the mixture was refluxed for 1 h in a 100-ml round-bottomed flask. The reaction mixture was poured on to crushed ice, and the solid separated was dried in air. The crude product was recrystallized from ethanol to yield pure product.

1-(2-Methyl-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2H)yl) ethanone (**11a**)

Yield: 67%; gray, solid; mp 236–238°C.

IR (KBr, cm^{-1}): 1288.49 (C–O), 1585.96 (C=N), 1604.83 (C=C), 1695.49 (C=O), 2928.04 (Me–CH), 3010.10 (Ar–CH).

^{1}H NMR ($CDCl_3$, δ ppm): 1.34 (d, 3H, CH_3), 2.40 (s, 3H, CH_3), 6.74–6.84 (q, 1H, CH), 8.30 (d, 2H, $J = 5.3$, Py), 8.76 (d, 2H, $J = 5.6$, Py).

1-(2-Phenyl-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2H)-yl) ethanone (**11b**)

Yield: 69%; brown, solid; mp 247–249°C.

IR (KBr, cm^{-1}): 1237.71 (C–O), 1581.68 (C=N), 1602.34 (C=C), 1688.48 (C=O), 2901.04 (Me–CH), 3010.10 (Ar–CH).

^1H NMR ($CDCl_3$, δ ppm): 2.27 (s, 3H, CH_3), 7.25 (s, 1H, CH), 7.62 (t, 2H, $J = 8.7$, Ar), 7.71 (t, 1H, $J = 8.8$, Ar), 7.83 (d, 2H, $J = 7.3$, Ar), 8.30 (d, 2H, $J = 5.4$, Py), 8.76 (d, 2H, $J = 5.6$, Py).

1-(2-(2-Chlorophenyl)-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2H)yl) ethanone (**11c**)

Yield: 65%; brown, solid; mp 250–252°C.

IR (KBr, cm^{-1}): 744.55 (C–Cl), 1303.92 (C–O), 1556.81 (C=N), 1596.61 (C=C), 1681.98 (C=O), 2983.98 (CH_3–CH), 3064.99 (Ar–CH).

^1H NMR ($CDCl_3$, δ ppm): 1.80 (s, 3H, CH_3), 7.24 (s, 1H, CH), 7.64 (t, 1H, $J = 8.7$, Ar), 7.71 (t, 1H, $J = 8.5$, Ar), 7.72 (d, 1H, $J = 7.4$, Ar), 7.82 (d, 1H, $J = 7.8$, Ar), 8.29 (d, 2H, $J = 5.6$, Py), 8.74 (d, 2H, $J = 5.8$, Py).

1-(2-(2-Nitrophenyl)-5-(pyridin-4-yl)-1,3,4-oxadiazol-3(2H)yl) ethanone (**11d**)

Yield: 68%; buff, solid; mp 257–259°C.

IR (KBr, cm^{-1}): 1271.13 (C–O), 1344.35, 1529.60 (C–NO_2), 1551.59 (C=N), 1600.61 (C=C), 1664.34 (C=O), 2945.98 (Me–CH), 3060.70 (Ar–CH).

^1H NMR ($CDCl_3$, δ ppm): 2.14 (s, 3H, CH_3), 7.24 (s, 1H, CH), 7.61 (t, 1H, $J = 8.7$, Ar), 7.70 (t, 1H, $J = 8.7$, Ar), 7.72 (d, 1H, $J = 7.8$, Ar), 7.84 (d, 1H, $J = 7.6$, Ar), 8.30 (d, 2H, $J = 5.4$, Py), 8.75 (d, 2H, $J = 5.6$, Py).

1-(2-Methyl-5-(pyridin-3-yl)-1,3,4-oxadiazol-3(2H)yl) ethanone (**12a**)

Yield: 67%; gray, solid; mp 121–123°C.

IR (KBr, cm^{-1}): 1282.71 (C–O), 1581.96 (C=N), 1602.23 (C=C), 1688.49 (C=O), 2901.04 (Me–CH), 3001.34 (Ar–CH).

^1H NMR ($CDCl_3$, δ ppm): 2.12 (d, 3H, CH_3), 2.32 (s, 3H, CH_3), 6.19–6.31 (q, 1H, CH), 8.10 (t, 1H, $J = 7.1$, Py), 8.29 (d, 1H, $J = 5.4$, Py), 8.71 (s, 1H, Py), 9.1 (d, 1H, $J = 5.6$, Py).

1-(2-Phenyl-5-(pyridin-3-yl)-1,3,4-oxadiazol-3(2H)-yl) ethanone (**12b**)

Yield: 69%; brown, solid; mp 122–124°C.

IR (KBr, cm^{-1}): 1282.71 (C–O), 1581.91 (C=N), 1602.54 (C=C), 1696.48 (C=O), 2901.04 (Me–CH), 3001.34 (Ar–CH).

^1H NMR ($CDCl_3$, δ ppm): 2.40 (s, 3H, CH_3), 6.52 (s, 1H, CH), 7.49–7.89 (m, 5H, Ar), 7.91 (d, 1H, $J = 5.4$, Py), 8.20 (t, 1H, $J = 7.2$, Py), 8.70 (d, 1H, $J = 5.6$, Py), 9.06 (s, 1H, Py).

1-(2-(2-Chlorophenyl)-5-(pyridin-3-yl)-1,3,4-oxadiazol-3(2H)yl) ethanone (**12c**)

Yield: 65%; brown, solid; mp 123–125°C.

IR (KBr, cm^{-1}): 750.33 (C–Cl), 1271.13 (C–O), 1582.54 (C=N), 1607.61 (C=C), 1691.98 (C=O), 3007.13 (Me–CH), 3068.85 (Ar–CH).

^1H NMR ($CDCl_3$, δ ppm): 2.40 (s, 3H, CH_3), 6.60 (s, 1H, CH), 7.49–7.89 (m, 4H, Ar), 7.91 (d, 1H, $J = 5.6$, Py), 8.20 (t, 1H, $J = 7.1$, Py), 8.70 (d, 1H, $J = 5.6$, Py), 9.46 (s, 1H, Py).

1-(2-(2-Nitrophenyl)-5-(pyridin-3-yl)-1,3,4-oxadiazol-3(2H)yl) ethanone (**12d**)

Yield: 69%; buff, solid; mp 123–125°C.

IR (KBr, cm^{-1}): 1305.85 (C–O), 1341.55, 1543.10 (C–NO_2), 1572.04 (C=N), 1597.61 (C=C), 1681.98 (C=O), 2985.91 (Me–CH), 3194.23 (Ar–CH).

^1H NMR ($CDCl_3$, δ ppm): 2.32 (s, 3H, CH_3), 7.25 (s, 1H, CH), 7.48–7.75 (m, 4H, Ar), 7.91 (d, 1H, $J = 5.6$, Py), 8.10 (t, 1H, $J = 7.2$, Py), 8.30 (d, 1H, $J = 5.6$, Py), 9.10 (s, 1H, Py).

Biologic activity

The synthesized compounds were evaluated for analgesic activity by the following tests:

Tail-flick test

Albino mice of either sex weighing 25–30 g were used for the test. They were divided into three groups each containing six mice. Each group was injected with test drug 25 mg/kg (i.p.) or vehicle (0.1% gum acacia solution) (i.p.). Pentazocine 17.5 mg/kg (i.p.) was used as reference. Mice were placed into cages leaving the tail exposed. Heat to the proximal third of the tail was applied. Within a few seconds the animal flicks the tail aside or tries to escape. The time until this reaction occurs was recorded by a stopwatch. The latency was recorded before and after 30 min (Fig. 2).

Hot-plate test

Three groups containing six albino mice of either sex each weighing 25–30 g were used for the test. Each group was injected with test drug 25 mg/kg (i.p.) or vehicle (0.1% gum acacia solution) (i.p.). Pentazocine 17.5 mg/kg (i.p.) was used as reference. A hot plate was maintained at 55° to 56°C. The animals were placed on the hot plate and the time until either licking or jumping occurs was recorded by a stop-watch. The latency was recorded before and after 30 min (Fig. 3).

Formalin-induced test

The albino mice of either sex each weighing 25–30 g were divided into three groups with each group containing six mice. Each group was injected with test drug 25 mg/kg (i.p.) or vehicle (0.1% gum acacia solution) (i.p.). Pentazocine 17.5 mg/kg (i.p.) was used as reference. Thirty min later, each group was injected with 0.025 ml of 5% formalin into the dorsal portion of the front paw. Each mouse was placed into a clear plastic cage for observation. Pain responses were indicated by elevation or favoring of the paw or excessive licking and biting of the paw. A biphasic response with an early (0–5 min) and a late (10–20 min) phase with high licking activity was observed (Fig. 4).

Antagonism effect of mecamylamine

The albino mice of either sex each weighing 25–30 g were divided into three groups each group containing six mice. Each group was injected with 2 mg/kg mecamylamine (i.p.), and 20 min later with test drug (**6**, **7a**, **7c**, **7d**, **8a**, **11a**, **12a**, and **12b**) 25 mg/kg (i.p.) or vehicle (0.1% gum acacia solution) (i.p.). Mice were placed into cages leaving the tail exposed. A heat to the proximal third of the tail was applied. Within a few seconds, the animal flicks the tail aside or tries to escape. The time until this reaction occurs was recorded by a stop-watch. The latency was recorded before and after 30 min (Fig. 5).

Statistical analysis

Results are expressed as mean ± SEM; *n* represents the number of animals. Data obtained from pharmacological experiments were analyzed by one-way analysis of variance (ANOVA) followed by Dunnet's multiple comparison test (dose–response and time course studies) and used to evaluate the results, employing Graph Pad Instat. A *P* value of less than 0.05 was considered statistically significant (Tables 1, 2, 3; Figs. 2, 3, 4, 5).

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