

ANALGESIC AND ANXIOLYTIC EVALUATION OF N'-SUBSTITUTED ARYLSULPHONYL AND BENZOYL DERIVATIVES OF 4-PYRIDINE CARBOHYDRAZIDE

SABAHAT NAEEM^{1*}, SHAMIM AKHTAR², NOUSHEEN MUSHTAQ², ARFA KAMIL³, IFFAT MAHMOOD⁴, SHAISTA ZAFAR², MUHAMMAD ARIF² AND Z.S. SAIFY⁵

¹Dow College of pharmacy, Dow University of Health sciences, Karachi, Pakistan

²Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Karachi, Pakistan

³Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Federal Urdu University of Arts, Science and Technology, Gulshan-e-Iqbal Campus, Karachi, Pakistan

⁴Department of Chemistry, Faculty of Science, Federal Urdu University of Arts, Science and Technology, Gulshan-e-Iqbal Campus, Karachi, Pakistan

⁵H.E.J. Research Institute of Chemistry, University of Karachi, Pakistan

Corresponding author e-mail: sabahatnaeem@hotmail.com

Abstract

4-Pyridine carbohydrazide (4-PCH) and its synthetic derivatives divulged an array of pharmacological activities. In continuation of our search for development of new potent and effective drug candidates, this study was aimed to investigate the previously synthesized N'-substituted arylsulphonyl and benzoyl derivatives of PCH for analgesic and anxiolytic effects by known experimental protocols. It was found that only N'-[(4-methylbenzene) sulfonyl]pyridine-4-carbohydrazide (1) exhibited analgesia at the dose of 25 mg/Kg body weight in tail flick test. In open field test (OFT), it was observed that N'-[(4-bromobenzene)sulfonyl]pyridine-4-carbohydrazide (3), N'-[(4-nitrobenzene)sulfonyl]pyridine-4-carbohydrazide (4) and N'-[4-methylbenzoyl]pyridine-4-carbohydrazide (5) showed anxiolytic like behavior. Structure Activity Relationship (SAR) was also ascertained.

Introduction

Pain is a multi-dynamic sensory experience, innately irritated and connected with ache and discomfort (Woolf, 2004), commonly classified as acute or chronic (Sessle, 2011). It is the most common cause of visiting a physician; study has reported that a 5th of the adult populace endure chronic pain in Western states (Oertel and Lotsch, 2013). Pain transmission is a compound mechanism, instigated by stimulation of nociceptors in periphery (Julius and Basbaum, 2001); often turns into centralized by maladaptive retorts inside the CNS which significantly change brain systems and hence behavior. Chronic pain thereby must be regarded as brain disorder (Borsook, 2012). Analgesics (drugs that disrupt nociceptive pathways) are broadly classified into NSAIDs and opioids. Both are associated with serious side effects (Becker and Phero, 2005; Slater *et al.*, 2010).

Anxiety is associated with sequential vague danger, a condition of persistent nervousness about the future damage, described by 'stress, negative affect and a sense of insecurity' (Grillon, 2008). Clinicians classified anxiety as normal and pathological (Belzung and Griebel, 2011). It has identified as a highly rified and chronic anarchy with inception during youth (Beesdo *et al.*, 2009), with a prevalence of 18.1% in United States (Kessler, 2005). The mechanism of anxiety is not completely understood but most of the studies have suggested the role of dysfunctional GABA neurotransmission (Nemerof, 2003a; Lydiard, 2003), besides some other neurotransmitters also involved including serotonin, nor-adrenaline and dopamine (Durant *et al.*, 2010). Anxiolytic agents that activate GABAergic system (Hoehn-Saric, 1982) include benzodiazepines and barbiturates, both possess various side effects (Nemeroff, 2003b).

Researchers have been exploring safe and efficient analgesics and anxiolytic agents for centuries. Based on the previous encouraging results (Naeem, 2014), we have undertaken to test our synthesized derivatives of PCH (Fig. 1) for potential effect on pain and anxiety.

Materials and Methods

Treatment of mice: Mice of either sex having weight 20-30g were purchased from Agha Khan University, Karachi and housed in cages with free admittance to food and water for three days prior to study.

The test compounds (Fig. 1) were N'-[(4-methylbenzene)sulfonyl]pyridine-4-carbohydrazide (1), N'-[(2,4,6-trimethylbenzene)sulfonyl]pyridine-4-carbohydrazide (2), N'-[(4-bromobenzene)sulfonyl]pyridine-4-carbohydrazide (3), N'-[(4-nitrobenzene)sulfonyl]pyridine-4-carbohydrazide (4) and N'-[4-methylbenzoyl]pyridine-4-carbohydrazide (5) and N'-[(3,5-dinitrobenzene) sulfonyl] pyridine-4-carbohydrazide (6).

Compounds 1-5 dissolved in WFI were administered via intraperitoneal route. Test compound **6** suspended in tragacanth was orally administered in the doses of 25mg/kg body weight. Animals receiving solvent always run parallel as a control group.

Evaluation of Analgesic activity: Analgesic response of the test compounds was examined following Tail flick method reported by di-Stasi et al. (di-Stasi *et al.*, 1988). During the study, each mice was held in an appropriate restrainer with entire tail (2-3cm marked) extending out. The marked area was dipped in a water bath (maintained at 51°C). The animals were observed for pre-drug and post drug latency time for a test period of 180min. Mean Tail Flick latency difference (TFLD) produced by test and standard drugs was calculated as: Analgesia TFLD = (post drug TFL – pre drug TFL).

Statistical Analysis: Analgesic activity was expressed as Mean TFLD \pm SEM in terms of seconds. Pethidine HCl was used as a positive control.

Evaluation of Anxiolytic Activity: Open field test is a screening method to monitor the exploration, locomotion and anxiety related behavior in rodents (Denenberg, 1969; Prut and Belzung, 2003). In the open field apparatus (area 76cm², with walls 42cm high and lines dividing the floor into 25 equal squares) each mice was allowed to walk around freely in 5min time interval and the number of square boxes crossed was counted. Number of rearing and assisted rearing was also monitored. An increase number of square crossing is indicative of anxiolytic-like effect (Yadav *et al.*, 2008; Thippeswamy *et al.*, 2011).

Results

The results of tail flick method are reported in Fig. 2 and that of open field activity are presented in Fig. 3. Fig. 2 showed a comparison of mean TFL values \pm SEM (in sec) produced by all the derivatives and the standard drug Pethidine HCl. It was observed that only compound **1** displayed appreciable analgesic profile with a earlier onset of action at 30min post drug administration (1.162 ± 0.097), reaching a maximum at 90min (2.853 ± 1.158) and continued till 150min (1.036 ± 0.031). Compound **3** also produced a slight relief in pain at 30min (1.542 ± 0.179) but after this the response was subsided.

Fig. 3 displayed mean number of square crossing (\pm SEM) produced by the control and test drugs in open field test. It was noticed that compounds **3**, **4** and **5** resulted in raised number of squares traversed with values 129.625 ± 11.127 , 180.31 ± 6.578 and 149.5 ± 9.317 respectively. A slight increment was also observed in rearing and assisted rearing for the same drugs.

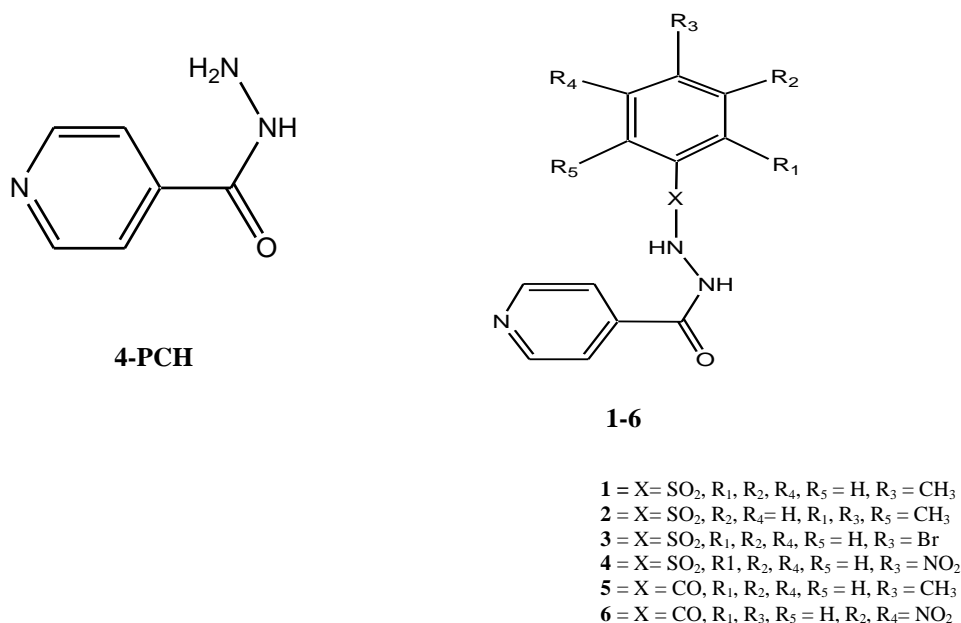
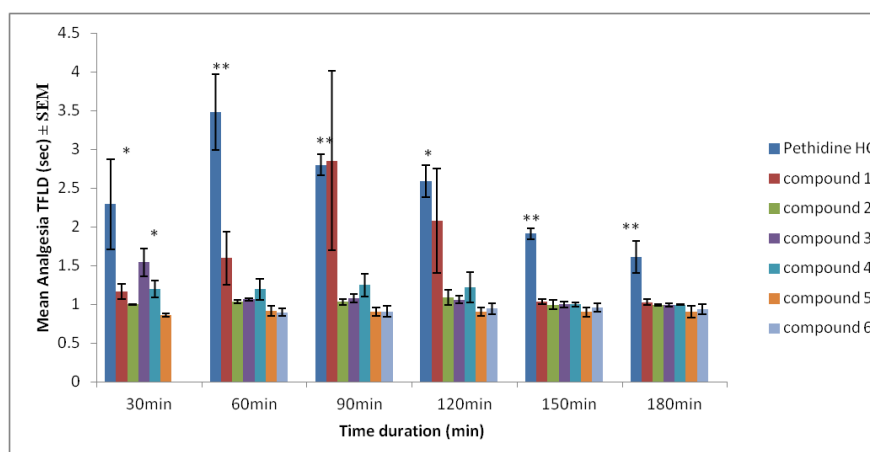
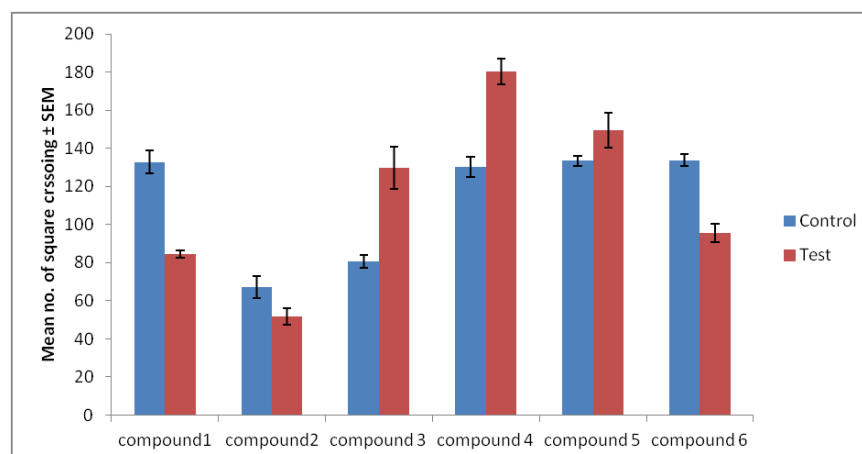


Fig. 1. N'-substituted derivatives of PCH.



Significant difference by Student t- test: * $p < 0.05$ and ** $p < 0.01$
Fig. 2. Comparison of Analgesic activity of Derivatives 1-6 and Pethidine HCl.



Significant difference by Student t-test: * $p < 0.05$ and ** $p < 0.01$
Fig. 3. Anxiolytic activity of Test Compounds 1-6 by open field test.

Discussion

The study was performed to explore the potential of the derivatives as analgesic and anxiolytic agents. Structure-activity-relationship (SAR) was also studied. The past evidence showed that the parent compound 4-PCH was associated with weak analgesia (Alder and Zbinden, 1973). The same molecule was responsible to cause reduction of gamma-aminobutyric acid (GABA) and hence worked as an anxiogenic drug (Lal and Harris, 1985; Carta *et al.*, 2008).

From the results of analgesic activity no noticeable antinociception was observed in the test derivatives except for arylsulphonyl compound 1 and 3 having methyl and bromo groups at para position. Introduction of benzoyl moiety remains dormant regardless of the substituents. This finding might suggest that the inclusion of bulkier ring with electron donating specie at para position managed to retain the analgesic behavior of the lead compound.

Results of the study showed that compound 3, 4 and 5 possessed anxiolytic-like effects. Derivatives 3 and 4 have *p*-bromo and *p*-nitro groups attached at arylsulphonyl ring respectively. Derivative 5 have *p*-methyl substituent at the benzoyl ring. This might explain that apart from the type of ring, substitution of para position is somewhere involved in reducing anxiety (except for compound 1).

Conclusion

From the study it has been concluded that the compound 1 possessed slight analgesic activity. Compound 3, 4 and 5 caused reduction in anxiety. These hearten results demand further investigation to establish a safe mechanism of action for the active derivatives that will support the development process of safe therapeutic molecules for anxiety and pain.

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