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Synthesis of novel 2-alkyl-4-yl-N-(5-thiophen-2-ylmethyl-[1,3,4]thiadiazol-2-yl)-acetamide derivatives as potent local anaesthetic agents

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ABSTRACT

Various 2-alkyl-4-yl-N-(5-thiophen-2-ylmethyl-[1,3,4]thiadiazol-2-yl)-acetamides (3a-f) were synthesized from condensation of secondary amines with 2-chloro-N-(5-thiophen-2-ylmethyl-[1,3,4]thiadiazol-2-yl)-acetamide(2).Structures of all the newly synthesized compounds were established by analytical and spectral data. All the newly synthesized final compounds were screened for their local anaesthetic activity using the rat sciatic nerve model.

Keywords: 2-amino [1,3,4] thiadiazole, Chloroacetylation, Local anaesthetic agents.

1. INTRODUCTION

Local anaesthetic agents are the compounds that block nerve fibre conduction when applied locally to nerve tissue in appropriate concentrations. They block the voltage-sensitive sodium channels of every type of nerve fibre blocking sodium entry into neuron thereby eliminating their action potential. [1] The great practical advantage of the local anaesthetics is that their action can be reversed when they are removed from the nerve tissue. In this case there is a complete recovery of the nerve function and the action potential, with no evidence of structural damage to nerve fibre or cells. [1]

The structural requirements [2,3] for a local anaesthetics are a lipophilic (aromatic) head, a hydrophilic end bearing a tertiary amine and an intermediate substituted alkyl chain. Compounds having an amino group linked to a heterocyclic nucleus, such as lipophilic moiety display greater activity & less toxicity than benzene analogues [4,5] Some derivatives of 2-aminothiazoles [6,7,8,9] are reported to possess local anaesthetic activity. In view of this we report here in synthesis of various thienyl substituted 2-alkyl-acetamide thiadiazole derivatives as potent local anaesthetic agents.

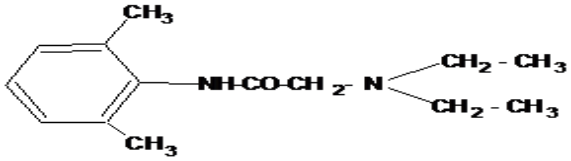
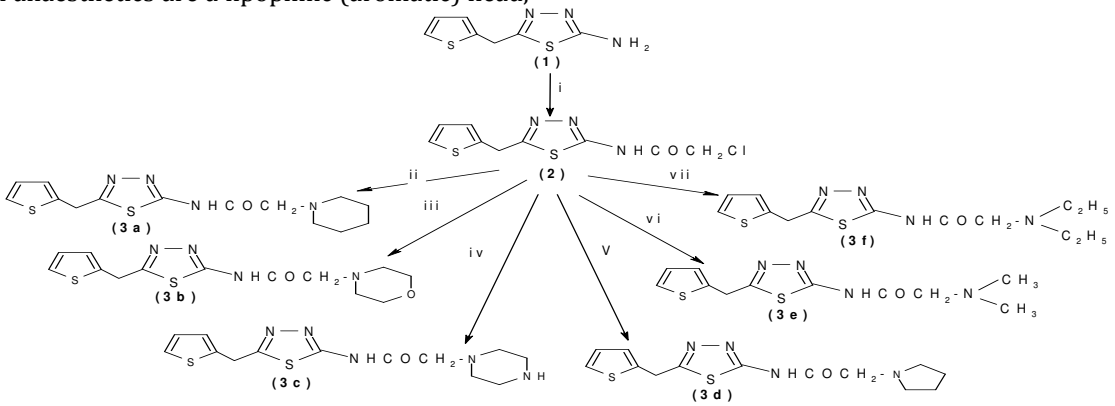


Figure - 1: Structure of lidocaine



Scheme - 1: Lidocaine derivatives.

1.1. Reagents and conditions

i: Glacial AcOH, Chloroacetyl chloride reflux.

ii: Piperidine, 1,4 dioxan reflux**.**

iii: Morpholine, 1,4 dioxan reflux.

iv: Piperazine,1,4 dioxan reflux**.**

v: Pyrrolidine,1,4 dioxan reflux**.**

vi: Dimethyl amine, 1,4 dioxan reflux.

vii: Diethylamine,1,4dioxan reflux.

2. EXPERIMENTAL

2.1. MATERIALS

All chemicals & reagents were purchased from Sigma-Aldrich Chemicals Pvt. Ltd India. Melting points were determined in open capillaries. The IR spectra were recorded on Nicolet Impact-FT-IR spectrophotometer,(Model-410,USA)using KBr pellet technique.^{1}HNMR experiments were performed on a 300 MHz Bruker AC-300F spectrometer (Model RX-300, Switzerland) using TMS as an internal standard in $CDCl_3$. All chemical shifts were reported as δ (ppm) values. All the newly synthesized compounds were analyzed for C, H, N and results were found to be within the range of ± 0.4% of the theoretical value.

2.2. Methodology

2.2.1. Synthesis of 5-thiophen-2-ylmethyl-[1,3,4]thiadiazol-2-ylamine (1)

A mixture of 2-thiophene acetic acid (0.1mole) and thiosemicarbazide (0.1mole) in phosphorousoxychloride (30 mL) was refluxed gently for 45 mins. The reaction mixture was cooled and quenched (highly exothermic) with cold water (90 mL). The resulting solution was refluxed for additional 4 hours and filtered hot. The filtrate was cooled and basified with aqueous potassium hydroxide solution. The solid that separated was filtered, washed with water, dried and recrystallized from ethanol.

2.2.2 Synthesis of 2-chloro-N-(5-thiophen-2-ylmethyl-[1,3,4]thiadiazol-2-yl)-acetamide (2)

To a suspension of 5-thiophen-2-ylmethyl-[1,3,4]thiadiazol-2-ylamine **(1)**(0.1mol) in glacial acetic acid (50 ml) was added chloroacetylchloride (0.1 mol) drop wise with stirring. The reaction mixture was then heated on steam bath for 3 h, cooled and poured into crushed ice (100gm). The solid that separated was filtered, washed with water, dried and recrystallized from ethanol.

2.2.3 Synthesis of 2-alkyl-4-yl-N-(5-thiophen-2-ylmethyl-[1,3,4]thiadiazol-2-yl)-acetamides. (3a-f)

A mixture of 2-chloro-N-[substituted thiadiazol-2-yl]acetamide **(2)** (0.1mol) and various secondary amines like piperidine, morpholine, piperazine, pyrrolidine, dimethylamine & diethylamine (0.1 mol) in 1,4 dioxan (40 ml) were refluxed for 4 h. The reaction mixture was cooled, poured into crushed ice (100gm). The solid that separated was filtered, washed with water, dried and recrystallized from ethanol to obtain solid **(3a-f)** respectively.

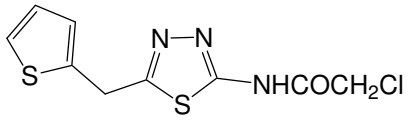
3. RESULTS AND DISCUSSION

Chloroacetylation of [1,3,4]thiadiazoles with chloroacetylchloride in the presence of glacial acetic acid leads to the formation of substituted thiadiazole acetamide derivatives **(2)** in excellent yields. Appearance of an absorption band at 1676 cm^{-1} due to amide carbonyl in IR and chemical shifts at around 12.51 δ ppm in ^{1}H NMR confirms –NH proton which is attached to the carbonyl carbon. Appearance of peak at δ 4.43 ppm (-$COCH_2$) further confirms formation of these compounds **(2).** Finally the target compounds have been synthesized by the condensation of (**2)** with different secondary amines like piperidine, morpholine, piperazine, pyrrolidine, dimethylamine & diethylamine in dioxan as solvent to get final compounds **(3a-f)** respectively.

In ^{1}H NMR spectra of morpholine derivatives (**3b**) two triplets (each for 4 protons) were observed at δ 2.58 (C_3, C_5-H; N-CH_2) and δ 3.72 (C_2, C_6-H; O-CH_2). For pyrrolidine derivatives (**3d**), two multiplets (in few cases two broad singlets) each for 4 protons were observed at δ 1.7 (C_3, C_4-H *i.e.* -CH_2-CH_2-) and δ 2.6 (C_2, C_5-H; N-CH_2) & for piperidine derivatives (**3a**), N-CH_2 (C_2, C_6) protons resonated at δ 2.4 as triplet or broad singlet for 4 protons and C_3, C_4 and C_5 protons resonated in the region δ 1.4-1.7 as multiplets for 6 protons. These analogues of lidocaine with diethylamino substitution showed two triplets at δ 1.14 (-N-CH_2-CH_3) indicating 6 protons and δ 2.67 (-N-CH_2-CH_3) indicating the presence of 4 protons which confirms the formation of these final compounds **(3f).** Similarly compounds with dimethylamino **(3e)** substitution showed respective chemical shifts in ^{1}H NMR spectra. All these final compounds were further confirmed by ^{13}C-NMR & by mass spectrum in which m/z of these final derivatives coincides with the molecular weight of the compounds.

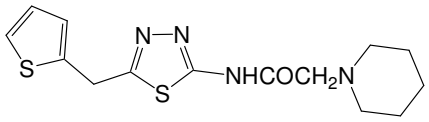
Thus the structures of all these newly synthesized compounds were established by their spectral and analytical data. These 2-amino thiadiazole analogues of lidocaine **(3a-f)** were tested *in vivo* for their local anaesthetic activity by rat sciatic nerve block.

3.1. Synthesis of 2-chloro-N-(5-thiophen-2-ylmethyl-[1,3,4]thiadiazol-2-yl)-acetamide (2).



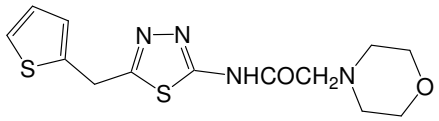
Brown solid (ethanol), yield 91%, mp 140-141^{0}C. IR (KBr) υ_{max} cm^{-1} : 3202, 3178, 1648, 1574. ^{1}H-NMR (300MHz ,$CDCl_3$) δ; 12.51 (1H, s, NH), 4.42 (2H, s, $COCH_2$), 4.36 (2H, s, CH_2), 7.02 - 7.28 (3H , m, Ar-H). Anal.Calcd. for: $C_9H_8ClN_3OS_2$; C, 39.49 ; H, 2.95 ; N, 15.35 %. Found: C, 39.40 ; H, 2.91 ; N, 15.32 %.

3.2. 2-piperidin-1-yl-N-(5-thiophen-2-ylmethyl -[1,3,4]thiadiazol-2-yl)-acetamide (3a)



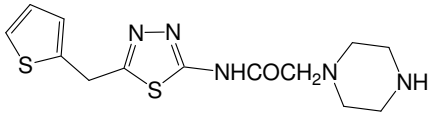
Brown solid (ethanol), yield 61%; mp 250 – 251^{0}C. IR (KBr) υ_{max} cm^{-1} : 3151, 2937, 2854, 1694, 1555. ^{1}H - NMR (300MHz, $CDCl_3$) δ; 12.05 (1H, s, NH), 1.54 -1.60 (6H, t, C_3, C_4, C_5-H, piperidine), 2.43-2.47 (4H, t, C_2, C_6-H, piperidine), 4.26 (2H, s, $COCH_2$), 3.11 (2H, s, CH_2),6.92 - 7.23 (3H, m, Ar-H). Anal.Calcd. for : $C_{14}H_{18}N_4OS_2$; C, 52.15 ; H, 5.63 ; N, 17.38 %. Found: C, 52.10 ; H, 5.61 ; N, 17.31 %.

3.3. 2-morpholin-4-yl-N-(5-thiophen-2-yl methyl-[1,3,4]thiadiazol-2-yl)-acetamide (3b)



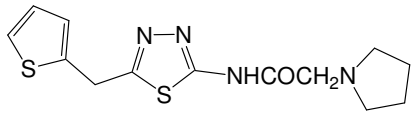
Brown solid (ethanol), yield 80%; mp 285 – 286^{0}C. IR (KBr) υ_{max} cm^{-1} : 3162, 2949, 2917, 1696, 1565. ^{1}H-NMR (300MHz, $CDCl_3$) δ; 12.45 (1H, s, NH), 2.61-2.64 (4H, t, C_3, C_5-H, morpholine), 3.77-3.80 (4H, t, C_2, C_6-H, morpholine), 4.35 (2H, s, $COCH_2$), 3.28(2H, s, CH_2), 7.01 - 7.28 (3H, m, Ar-H). Anal.Calcd. for : $C_{13}H_{16}N_4O_2S_2$; C, 48.13 ; H, 4.97 ; N, 17.27 %. Found: C, 48.10 ; H, 4.91 ; N, 17.24 %.

3.3. 2-piperazin-1-yl-N-(5-thiophen-2-ylmethyl -[1,3,4]thiadiazol-2-yl)-acetamide (3c)



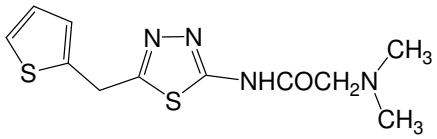
Brown solid (ethanol), yield 61%; mp 230 – 231^{0}C. IR (KBr) υ_{max} cm^{-1} : 3256, 3109, 2935, 2994, 1648, 1519. ^{1}H - NMR (300MHz, $CDCl_3$) δ; 12.05 (1H, s, NHCO), 2.41 -2.60 (8H, m,piperazine), 5.1 (1H, s, N-H, piperazine), 4.26 (2H, s, $COCH_2$), 3.11 (2H, s, CH_2),6.92 - 7.23 (3H, m, Ar-H). Anal.Calcd. for : $C_{13}H_{17}N_5OS_2$; C, 48.27 ; H, 5.30 ; N, 21.65 %. Found: C, 48.25 ; H, 5.26 ; N, 21.62%.

3.4. 2-pyrrolidin-1-yl-N-(5-thiophen-2-ylmethyl-[1,3,4]thiadiazol-2-yl)-acetamide (3d)



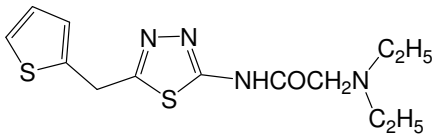
Brown solid (ethanol), yield 61%; mp 178 – 179^{0}C. IR (KBr) υ_{max} cm^{-1} : 3150, 2935, 2854, 1691, 1570. ^{1}H - NMR (300MHz, $CDCl_3$) δ; 12.05 (1H, s, NH), 1.82 (m, 4H, C_3, C_4-H, pyrrolidine), 2.60 (m, 4H, C_2, C_5-H, pyrrolidine), 4.26 (2H, s, $COCH_2$), 3.11 (2H, s, CH_2),6.92 - 7.23 (3H, m, Ar-H). Anal.Calcd. for : $C_{13}H_{16}N_4OS_2$; C, 50.62 ; H, 5.23 ; N, 18.17 %. Found: C, 50.60 ; H, 5.21 ; N, 18.11 %.

3.5. 2-Dimethylamino-N-(5-thiophen-2-ylmethyl-[1,3,4]thiadiazol-2-yl)-acetamide (3e)



Brown solid (ethanol), yield 71%; mp 174 – 175^{0}C. IR (KBr) υ_{max} cm^{-1} : 3170, 3065, 2980, 2864, 1688, 1555. ^{1}H-NMR (300MHz, $CDCl_3$) δ; 12.45 (1H, s, NH), 2.29 (6H, s, N- CH_3-CH_3), 4.2 (2H, s, $COCH_2$), 3.16(2H, s, CH_2) ,6.91 - 7.21(3H, m, Ar-H). Anal.Calcd. for: $C_{11}H_{14}N_4OS_2$; C, 46.79 ; H, 5.00 ; N, 19.84 %. Found: C, 46.75 ; H, 5.01 ; N, 19.81 %.

3.6. 2-Diethylamino-N-(5-thiophen-2-ylmethyl-[1,3,4]thiadiazol-2-yl)-acetamide (3f)



Brown solid (ethanol), yield 71%; mp 261 – 262^{0}C. IR (KBr) υ_{max} cm^{-1} : 3170, 3065, 2980, 2864, 1688, 1555. ^{1}H-NMR (300MHz, $CDCl_3$) δ; 12.45 (1H, s, NH), 1.10 -1.12 (6H, t, N- CH_2-CH_3), 2.56 - 2.60 (4H, t, N- CH_2-CH_3), 4.2 (2H, s, $COCH_2$), 3.16(2H, s, CH_2) ,6.91 - 7.21(3H, m, Ar-H). Anal.Calcd. for: $C_{13}H_{18}N_4OS_2$; C, 50.30 ; H, 5.84 ; N, 18.05 %. Found: C, 50.26 ; H, 5.81 ; N, 18.02 %.

3.7. Pharmacology

3.7.1 Rat sciatic nerve block

Triplicate sets of three groups of three male Wistar rats (weighing 180-200 g) were used [10] for the experiment. Aqueous solution of the test compound (2%, 0.2ml) was injected in each rat into the posterior aspect of the femur head. A complete loss of motor control of the injected limb

indicates a positive effect of the drug. The animals were observed to asses the duration of effect, from the time of onset of the motor paralysis at 5 min intervals for the first 30 min, and later 15 min intervals up to the first sign of motor activity.

Table – 1: Duration of local anaesthetic activity in rat sciatic nerve block.

Compound	Duration * (min)	
	1%	2%
3a	90 (±3.6)	210(±16.9)
3b	45 (±6.8)	52 (±6.9)
3c	25 (±10.5)	45 (±17.0)
3d	60 (±15.0)	180 (±18.2)
3e	55 (±11.0)	160 (±15.3)
3f	60 (±12.0)	160 (±20.3)
Lidocaine HCl	**65 (±10.7)**	**117 (±11.0)**

* In vivo duration of local anaesthetic activity in rat sciatic nerveblock (each rat received 0.2 mL of 1% and 2% anaesthetic solution).The values are means of ± SE of three determinations.

Table -2: Rabbit corneal anaesthetic activities

Compound	Corneal anaesthsia[1]
3a	92.3 ± 3.2
3b	54.2 ± 4.3
3c	44.3 ± 4.3
3d	86.6 ± 3.3
3e	78.8 ± 5.5
3f	82.6 ± 6.2
Lidocaine HCl[2]	**100**

[1]All compounds were in aqueous solution at 2% concentration. The values expressed as % of the anaesthetic activity of lidocaine (=100), are means ± SE of three determinations.

[2]Lidocaine hydrochloride was used as reference.

3.7.2 Corneal anaesthesia

Male Newzeland rabbits (weighing 2.5-2.7 kg) were used for this study. Local surface anesthesia was evaluated by determining the number of stimuli to the cornea every 3 min [10]. This was affected rhythmically with a Frey's horse-hair, in order to produce the blink reflex. Anesthetic effect was considered total, if the reflex did not occur after 100 stimulations. Care was taken at the beginning of the experiment to ascertain that this reflex was normal in both eyes of the rabbits used. The aqueous solutions (2%) of the compounds studied were dropped into the conjuctival sac so that the space between the eyelids contained a clearly visible film of solution for the set time of 3 min. Lidocaine solution (2%) was used as reference.

4. CONCLUSIONS

For all the tested compounds, it appears that the position and the nature of the side chain affect surface and infiltration activities. Thus in order to evaluate local anaesthetic activity, additional investigation also been conducted along with rat sciatic nerve block assay in comparison with the reference drug Lidocaine (Table 2). When a 2% solution was used several compounds (Table 1) exhibited better activity in blocking the rat sciatic nerve with respect to Lidocaine (160, 210 and 180 min.for motor activity recovering, respectively, versus 117 min.for lidocaine). It has been confirmed that reducing the ring structure with minimum number of hetero atoms & lengthening of terminal alkyl chain results in improvement of local anaesthetic activity. Analogues with a terminal piperidine, pyrrolidine & diethylamino substitutions displayed higher activity.

5. REFERENCES

1. Catterall W and Mackie K **Local anaesthetics**. In: Hardman JG, Limbird LE, Gilman AG (eds) Goodman and Gilman's **The pharmacological basis of therapeutics**, McGraw-Hill, New York, 10th ed: 2001; 687–731.
2. Lofgren N, **Studies on Local Anaesthetics**: Xylocaine A new synthetic drug. Hoegsstroms, Stockholm. 1948.
3. TakmanBH, CamougisG and LofgrenNM. Studies on local anaesthetics. Hydroxyalkylamioacylanilines as local anaesthetics. **Acta Pharm Suec.** 1969; 6(1): 25–32.
4. Bhargava PN and Chaurasia MR. New local anesthetics. Derivatives of 5-diethylaminoacetamido-2-arylimino-3-aryl-4-thiazolidones. **J. Pharm Sci.** 1969; 58: 896–898.
5. Mattocks AM and Hutchinson ON. Local anesthetics; N-dialkylaminoalkylimides of naphthalic and diphenylmaleic acids. **J. Am Chem Soc.** 1948; 70: 3474–3475.
6. Bhargava PN and Singh PR. Synthesis of new local anaesthetics II. **J. Indian Chem Soc.** 1960; 37: 241–243.
7. Srivastava PN and Rai SK. Some derivatives of 2-aminothiazole as potent local anaesthetic. **Eur J. Med Chem Chim Ther.** 1980; 15: 274.

8. Lakhan R and Rai BJ. Local anaesthetics. IV. Synthesis and activity of 2-(N-substituted or N,N disubstituted aminoacetamido)-4- or -4-5-substituted thiazoles. **Farmaco Sci.** 1986; 41(10): 788–793.
9. Bhargava PN, Prakash S and Singh HD. Synthesis of 2-N,N dialkyl (or alkyl aryl) aminoacetamido-(substituted)-benzothiazoles or -thiazoles as local anaesthetics. **J. Indian Chem Soc.** 1978; 55(7): 726–729.
10. Caliendol G, Di Carloz R, Grecol G, Griecol P, Meli R, Novellinol E, Perissuttil E, and Santagada V. Synthesis, local anesthetic activity and QSAR studies for a set of N-[2-(alkylamino)ethyl]benzotriazol-x-y1 acetamides**. Eur J Med Chem.** 1995; 30: 603–608.