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(54) **6,7-dihydro-[1,3,4]thiadiazolo-[3,2-a][1,3]diazepin derivatives and pharmaceutical composition containing the same as neuromuscular blocker or skeletal muscle relaxant, and method for the preparation**

6,7-Dihydro-[1,3,4]thiadiazolo-[3,2-a][1,3]diazepin-Derivate und pharmazeutische Zusammensetzungen damit als Schlaf- oder Anästhesiemittel und Herstellungsverfahren dafür

Dérivés de la 6,7-dihydro-[1,3,4]thiadiazolo-[3,2-a][1,3]diazepine et compositions pharmaceutiques contenant ceux-ci en tant qu'agent hypnotique ou anesthésique et procédé pour leur préparation

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Description

[0001] The present invention relates to compounds useful as neuromuscular blocker or skeletal muscle relaxant, pharmaceutical compositions containing the compound as well as a method for the preparation.

[0002] Neuromuscular blockade and anaesthesia are essential for surgical procedures. Muscle relaxation is required for the ease of surgical operations. The use of neuromuscular blockers or skeletal muscle relaxants will decrease the doses of the general anaesthetics required to induce consciousness in addition to muscle relaxation. Beside their adjunct use in anaesthesia, neuromuscular blockers are used in hospitals to ease tracheal intubation of the patients and to suppress the patient's spontaneous breathing. Other uses of skeletal muscle relaxants include prevention of fractures during electroshock therapy, suppression of titanic convulsions and diagnosis of myasthenia gravis. The medical history of these useful drugs dates back to the introduction of curare, the alkaloidal extract of *Chardrodendron tomentosum.* This was followed by the introduction of Gallamine, Succinylcholine (suxa-methonium), Pancuronium, Alcuronium and Atracurium (Singh, M. et al, J. Chem. Pharm. Res. 2010, 2, 264-273).

[0003] Neuromuscular blockers can be classified according to their mechanism of action into competitive neuromuscular blockers and depolarizing blockers. Most of the agents currently in use are of the competitive type. Competitive neuromuscular blockers block the nicotinic receptors located postsynaptic on skeletal muscle membranes. They compete with acetylcholine (ACh) for these receptors and prevent its action on evoking muscular contraction and thus muscle relaxation ensues gradually. Competitive neuromuscular blockers include d-Tubocurarine, Gallamine, Atracurium, Pancuronium, Vercuronium, Mevacurium and others. The depolarizing blockers mimic ACh in their ability to activate the postsynaptic nicotinic receptors on the skeletal muscles but differ from ACh in their ability to induce persistent depolarization of the skeletal muscles rendering them insensitive to any released ACh. The classical example of these drugs is Decamethonium which is not used in medicine now due to its prolonged action and the absence of a substance to reverse its action following operations. The only drug of this group that is still in use is Succinylcholine (Suxamethonium) due to its rapid action and short duration (up to 5 minutes following bolus injections at 0.5-2 mg/kg i.v.). Neuromuscular blockers can be classified according to onset of action and duration time into: ultra-short acting with onset within a minute following bolus i.v. administration and duration of 5 minutes e.g. Succinylcholine, and Gantacuriurn (Bigham, E. et al, US Patent 2001, No. 4179507); short acting with onset within 2 minutes and duration up to 20 minutes e.g. Mevacurium and Rocuronium; intermediate acting with onset within 5 minutes and duration up to 60 minutes e.g. Atracurium, Pancuronium, Vercuronium, Cisatracurium; long acting with onset up to 6 minutes and duration 75-100 minutes e.g. Pipercuronium and Doxacurium (Hunter, J. New Engl. J. Med. 1995, 332, 1691-1699; Omoigui E. The Anethesia Drugs Hand Book. 1995, Mosby, St. Louis, MO, USA).

[0004] The competitive neuromuscular blockers are mostly used in medicine due to their antagonism by anticholinesterases e.g. Neostigmine, Pyridostigmine or Edrophonium following the end of the surgical operations or tracheal incubations. However, those suffer from various side effects e.g. apneas and even non-neuromuscular blockade induced side effects. These include release of histamine with consequent hypotension, broncho-constriction and excessive mucus secretions, headache as observed with Atracurium, d-Tubocurarine, and Mevacurium, (Basta, S. et al, Br. J. Anaesth. 1983, 55, 105S-106S; El Bradie, S. J. Egypt Natl. Canc. Inst. 2004, 16, 107-113; Jooste, E. et al, Anesthesiology 2007, 106, 763-772), blockade of muscarinic M_2 cardiac receptors and parasympathetic ganglia with the ultimate induction of tachycardia and elevation of the arterial blood pressure as observed with Gallamine (Bigham, E. et al, US Patent 2001, No. 4179507), stimulation of sympathetic autonomic ganglia, induction of tachycardia, elevation of the arterial pressure, increase in the intraocular pressure and induction of hyperkalemia as observed with Succinylcholine (Hunter, J. New Engl. J. Med. 1995, 332, 1691-1699; Bigham, E. et al, US Patent 2001, No. 4179507). An ideal neuromuscular blocker should possess a rapid onset of action, reasonable duration, and rapid reversibility after ending its use together with freedom from non-neuromuscular blockade side effects.

[0005] Neuromuscular blockers are, for example, also known from DE 103 20 732 which are based on thiazolo-[3,2-a][1,3]diazepin derivatives.

[0006] Pollard B. J. Anaesthesia and Intensive Care Medicine, 2005, 6, 189-192 discusses a variety of neuromuscular blocking agents and reversal agents.

[0007] It is an object of the present invention to provide a neuromuscular blocker or skeletal muscle relaxant which overcomes the drawbacks of the prior art. Especially a compound shall be provided showing a competitive neuromuscular blocking activity, with reasonable onset of action and intermediate duration which can be rapidly reversed by anticholinesterases. Additionally, a pharmaceutical composition containing such an agent shall be provided, as well as a method for its preparation.

[0008] The objects are achieved by the features of the independent claims. Preferred embodiments are disclosed in the sub-claims.

[0009] The term "alkyl" with regard to the definition of R_1-R_4 in the compound according to formula 1 is to be understood to comprise linear and branched alkyls. The term "halo" shall comprise derivatives which are mono-, di-, tri- or polyhalosubstituted.

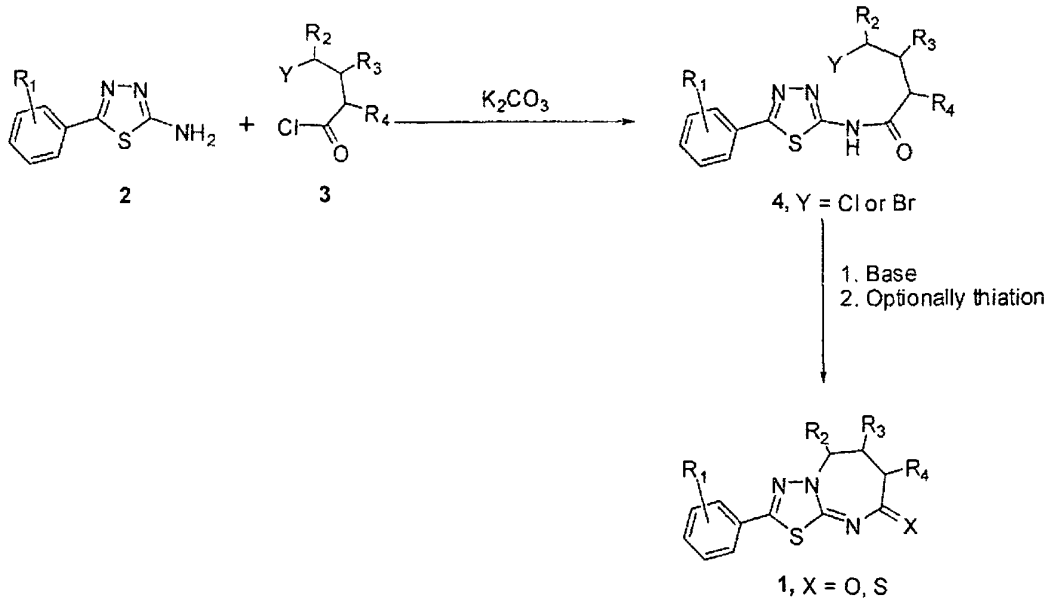
[0010] If possible, all substituents R_1-R_4 may be optionally further substituted, for example by halogen, amino, substituted amino, C_1-C_{20}-alkyl, C_1-C_{20}-haloalkyl, C_1-C_{20}-alkoxy or C_1-C_{20}-haloalkoxy, or mercapto, alkylthio, alkylamino, arylthio, heteroarylthio, arylamino or heteroarylamino.

[0011] In one embodiment, at least two substituents R_1-R_4, preferably R_3 and R_4 may be taken together to form an, optionally substituted, alicyclic, aryl or heteroaryl ring system.

[0012] Surprisingly, it was found that the compounds as proposed in the present invention overcome many of the disadvantages and problems that are usually accompanied with neuromuscular blockers known in the art. Especially the compounds of the present invention show a comparable competitive neuromuscular blocking activity, with reasonable onset of action. Also, intermediate duration which can be rapidly reversed by anticholinesterases is acceptable.

[0013] 1,3,4-Thiadiazolo[3,2-a][1,3]diazepine analogs could be obtained adopting published methods (Molina, P. et al, J. Org. Chem. 1993, 58, 5264-5270; Imming, P. et al, Arch. Pharm. (Wienheim) 1995, 238, 207-215). The compounds of invention and their analogs (**1**) are synthesized according to an inventive method, Scheme 1.

Scheme 1



[0014] The proper 2-amino-5-substituted-1,3,4-thiadiazole (**2**) were acylated with the suitable acid chloride derivatives (**3**), where Y is chlorine or bromine, preferably bromine, and anhydrous potassium carbonate in a suitable solvent, such as, for example, toluene, ethylbenzene, o-, m-, and p-xylene, octane, nonane and isopropylbenzene, preferably toluene and ethylbenzene at temperature ranging from about 100° to 150°C, preferably 100-120°C. The products **4** can be purified by silica gel and neutral alumina chromatography. Compounds of the formula **4** were cyclized using secondary amines, such as for example, diethylamine, pyrrolidine, morpholine, piperidine, N-methylpiperazine, preferably pyrrolidine and piperidine, in a suitable solvent, such as for example toluene, ethylbenzene, o-, m-, and p-xylene, isopropylbenzene, preferably toluene, o-xylene at temperature ranging 100-180°C, preferably 120-130°C. The products of the formula **1** were obtained and purified using either silica gel or alumina column chromatography. Compounds of formula **1** can be optionally thiated in using conventional thiating agents, for example, Lawesson-reagent, according to report methods, such as Nishio T. et al., Tetrahedron 1999, 55, 5017-5026; Swanson D.M. et al., Eur. J. Med. Chem. 2009, 44, 4413-4425.

[0015] Adult male Wistar rats (250 g) and 4-day old chicks (50 g) were used to conduct the neuromuscular blocking activity evaluation. They were housed in cages and kept at a temperature of 20 ± 2°C and a relative humidity of 55 ± 5% with a light-dark cycle of 12 h. The animals were provided with Purina rodent's chow pellets supplied by Grain silos and Flour Mills Organization, Riyadh, Saudi Arabia and had both food and water *ad libitum*. The chicks were bought from the local market in Riyadh. Male Wistar rats (250 g) were randomly divided into various groups (N = 4-8 animals). The animals were prepared following modifications of the reported method (Henning, R. Br. J. Pharmacol. 1993, 108, 717-720; Thesleff, S. et al, J Pharmacol. Expt. Ther. 1954, 111, 99-118). Test drugs, standards or vehicles were administered intraperitoneal dissolved in dimethyl sulfoxide, or water as appropriate in various doses 1-100 mg/kg. In all experiments a control group was included and received intraperitoneal injections of the test compounds' vehicle. The

ED_{50} (Effective dose required to produce 50% reduction of the rat tibialis twitch) was calculated for each drug. Also the dose that produced 90% of twitches depression was calculated. The response of the muscle to titanic stimulation was tested before administration of any drug and following 90% inhibition of the twitches.

[0016] Administration of compounds of the formula **1** induced dose-dependent inhibitions of the twitches in rats i.e. neuromuscular blockade leads to skeletal muscle relaxation. The competitive neuromuscular blocker Atracurium and the depolarizing neuromuscular blocker Succinylcholine were used as positive controls. The ED_{50} values, times for onset for 50% and 90% inhibitions, the effects of tetanus, the anticholinesterase - Physostigmine 100% reversal, and the duration of the blocks were recorded. The onset times of compounds of the formula **1** were ranged from 3-10 minutes. The ED_{50} values were ranged from 0.15-0.36 mmoles/kg i.p. Representative example is shown in Example 3.

[0017] Intraperitoneal administration of compounds of the formula **1** in single doses ranged from of 0.15-0.3 mmoles/kg, induced flaccid paralysis of the chicks within 1-15 minutes. The paralysis started with head movement and drop; then complete flaccid paralysis occurred. The onset of head drops were ranged from 15 sec-11 minutes whereas the flaccid paralysis ranged from 1-15 minutes following administration. Atracurium and Succinylcholine were used as positive controls. The mechanism of action of compounds of the formula **1** was similar but with more rapid onset of action than that of Atracurium; and almost similar to that of Succinylcholine. Representative example is shown in Example 4.

[0018] Compounds of the invention (formula **1**) showed neuromuscular blocking activity on rats and chicks. The results obtained on rats following tetanus application during compounds of invention induced neuromuscular blockade suggest that the compounds of formula **1** acted via competitive mechanism with ACh released following electrical stimulation of the sciatic nerve to the postsynaptic nicotinic receptors on the tibialis muscle membrane. Such a competitive mechanism is supported by the studies in chicks in which compounds of the invention induced head drop and flaccid paralysis of the limbs, by the use of reported techniques (Thesleff, S. et al, J. Pharmacol. Expt. Ther. 1954, 111, 99-118). The complete reversal of compounds of the formula **1** induced block by the anticholinesterase - physostigmine confirms their competitive mechanisms of action. Competitive blockers-induced blockade is usually reversed by various anticholinesterases such as Neostigmine, Edrophonium and Pyridostigmine (Henning, R. Br. J. Pharmacol. 1993, 108, 717-720; Sacan, O. et al, Anesth. Analg. 2007, 104, 569-574; Garg, R. et al, J. Anesth. 2008, 18, 1-5; Hunter, J. et al, Br. J. Anaesth. 2006, 97, 123-126). Regarding the onset of action and duration, the studies in rats and chicks revealed that compounds of the formula **1** possess onset of action more rapid than the well established competitive neuromuscular blocker-Atracurium. The duration of compounds of the formula **1**-induced block is in the same range of the standard competitive neuromuscular blockers, such as Atracurium, Pancuronium, Veracuronium and Cisatracurium (Hunter, J. New Engl. J. Med. 1995, 332, 1691-1699; Omoigui E. The Anethesia Drugs Hand Book. 1995, Mosby, St. Louis, MO, USA).

[0019] The LD_{50} values of compounds of formula **1** were performed. Compounds were given intraperitoneally in doses ranging from 0.1-5 mmole/kg. The animals were observed for up to 6 hours continuously and were then kept under observation for 72 hours. All behavioral changes and death during the observation periods were recorded. The percentage of death at each dose level was then calculated, and the LD_{50} values were obtained (Ghosh, M., Fundamentals of Experimental Pharmacology, Scientific Book Agency, Calcutta. 1984, pp 153-158, 187-189). The Therapeutic Index of each compound was calculated following the determination of the effective neuromuscular blocking dose. Representative example is shown in Example 5.

[0020] The biological evaluation of the new compounds of the formula **1** of the invention revealed that the compounds are neuromuscular blockers which mark the era of the introduction of a new class of intermediate acting skeletal muscle relaxants. Compounds of this invention acted via competitive mechanism with ACh released which could be completely reversed by the anticholinesterase - Physostigmine. Therefore, compounds of the formula **1** of the invention have the potential use as muscle relaxants.

[0021] Compounds of the formula **1** of the invention, and their acid addition salts display neuromuscular blocking skeletal muscle relaxants activity. The present invention includes pharmaceutical formulations which, in addition to non-toxic, inert pharmaceutically suitable excipients, contain one or more active compounds according to the invention, or which consist of one or more active compounds according to the invention, as well as processes for the preparation of these formulations.

[0022] The present invention also includes pharmaceutical formulations in dosage units. This means that the formulations are in the form of individual parts, for example tablets, dragees, capsules, pills, and ampoules, of which the content of active compound corresponds to a fraction or a multiple of an individual dose. The dosage units can contain, for example, 1, 2, 3 or 4 individual doses or 1/2, 1/3 or 1/4 of an individual dose. An individual dose preferably contains the amount of active compound which is given in one administration and which usually corresponds to a whole, a half, a third or a quarter of a daily dose.

[0023] By non-toxic, inert pharmaceutically suitable excipients there are to be understood solid, semi-solid or liquid diluents, fillers and formulations auxiliaries of every kind.

[0024] Tablets, dragees, capsules, pills, granules, solutions and sprays may be mentioned as preferred pharmaceutical formulations.

[0025] Tablets, dragees, capsules and pills can contain the active compound or compounds alongside the customary excipients, such as (a) fillers and extenders, for example starches, lactose, sucrose, glucose, mannitol and silica, (b) binders, for example carboxymethylcellulose, alginates, gelatin and polyvinylpyrrolidone, (c) humectants, for example agar-agar, calcium carbonate and sodium bicarbonate, (e) solution retarders, for example paraffin, and (f) resorption accelerators, for example quaternary ammonium compounds (g) wetting agents, for example cetyl alcohol and glycerol monostearate, (h) adsorbents for example kaolin and bentonite, and (i) lubricants, for example talc, calcium stearate and magnesium stearate and solid polyethylene glycols, or mixtures of the compounds listed under (a) to (i).

[0026] The tablets, dragees, capsules and pills can be provided with the customary coatings and shells, optionally containing pacifying agents, and can also be of such composition that they release the active compound or compounds only, or preferentially, in a certain part of the intestinal tract, optionally in a delayed manner, examples of embedding compositions which can be used being polymeric substances and waxes.

[0027] The active compound or compounds, optionally together with one or more of the above mentioned excipients could also be in a micro-encapsulate form.

[0028] Solutions and emulsions for parenteral administration can contain, in addition to the active compound or compounds, the customary excipients, such as solvents, solubilizing agents and emulsifiers, for example water, ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils, especially cotton seed oil, groundnut oil, maize germ oil, olive oil, castor oil and sesame oil, glycerol, glycerol-formal, tetrahydrofurfuryl alcohol, polyethylene glycol and fatty acid esters of sorbitol, or mixtures of these substances, in a sterile form which is isotonic with blood.

[0029] The therapeutically active compounds should preferably be present in the above-mentioned pharmaceutical formulations in a concentration of about 0.1 to 99.5, preferably of about 0.5 to 95% by weight of the total mixture.

[0030] The above-mentioned pharmaceutical formulations can also contain other pharmaceutical formulations; can also contain other pharmaceutical active compounds in addition to the active compounds according to the invention.

[0031] The above-mentioned pharmaceutical formulations are prepared in the customary manner according to known methods, for example by mixing the active compound or compounds with the excipient or excipients.

[0032] The present invention also includes the use of the active compounds according to the invention, and of pharmaceutical formulations which contain one or more active compounds according to the invention in human and veterinary medicine.

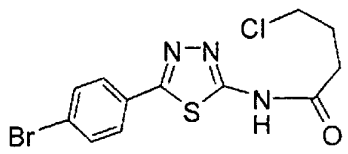
[0033] The actual dosage unit will be determined by such generally recognized factors as body weight of the patient and/or severity and type of pathological condition the patient might be suffering. With these considerations in mind, the dosage unit for a particular patient can be readily determined by the medical practitioner in accordance with the techniques known in the medical arts.

[0034] The precise instructions for pharmaceutical administration of the compounds and agents according to the invention necessarily depend on the requirements of the individual case, the nature of treatment, and of course the opinion of the treating physician.

Example 1

N-[5-(4-bromophenyl)-1,3,4-thiadiazol-2-yl]-4-chlorobutanamide

[0035]

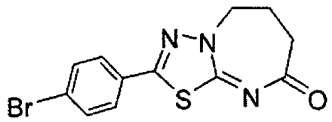


[0036] A mixture of 5-(4-bromophenyl)-1,3,4-thiadiazol-2-amine (10.2 g, 0.04 mol), 4-chloro-butyryl chloride (11.3 g, 9.0 ml, 0.08 mol) and potassium carbonate (5.5 g, 0.04 mole) in toluene (100 ml) was heated under reflux for 4 hr. The toluene was then evaporated under reduced pressure. The residue was then quenched with water, stirred, and filtered. The solid obtained was washed, dried and recrystallized from toluene to give the required product (12.5 g, 87% yield), mp 187-9°C, m/e 360, 15.7% (consistent with molecular formula $C_{12}H_{11}BrClN_3OS$, calcd. 360.66). ^1H NMR (DMSO-d_6): δ 1.96-2.22 (m, 2H, -CH_2), 2.69-2.81 (m, 2H, -CH_2), 3.71-3.72 (m, 2H, -CH_2), 7.49-7.88 (dd, 4H, ArH), 12.76 (br s, 1H, NH). ^{13}C NMR: δ 27.3, 32.1, 44.6, 123.8, 128.7, 129.4, 132.3, 158.5, 160.7, 170.7.

Example 2

(E)-2-(4-bromophenyl)-6,7-dihydro-[1,3,4]thiadiazolo[3,2-*a*][1,3]diazepin-8(5*H*)-one (GS-53)

[0037]



[0038] A mixture of N-[5-(4-bromophenyl)-1,3,4-thiadiazol-2-yl]-4-chlorobutan-amide (1.44 g, 0.004 mol) and piperidine (0.7 g, 0.8 ml, 0.008 mol) in toluene (50 ml) was heated under reflux for 3 h. The reaction mixture was cooled, poured into water and stirred. Toluene was separated dried and evaporated to give a crude product which was purified by repeated silica gel and neutral alumina column chromatography eluting with EtOAc/hexane (50:50 v/v) and $CHCl_3$/hexane (80:20 v/v); mp 204-7°C, m/e 324, 78% (consistent with molecular formula $C_{12}H_{10}BrN_3OS$, calcd. 324.20) ^1H NMR $CDCl_3$): δ 2.32-2.40 (m, 2H, $-CH_2$), 2.76 (t, *J* = 7.5 Hz, 2H, $-CH_2$), 4.29 (t, *J* = 7.5 Hz, 2H, $-CH_2$), 7.62-7.84 (dd, *J* = 8.5 Hz, 4H, ArH). ^{13}C NMR: δ 18.3, 31.2, 47.9, 125.0, 128.7, 129.5, 132.3, 157.5, 162.9, 173.7.

[0039] The NMR spectral data assignments of compounds of Example 1 and Example 2 are based on analysis of the ^1H, Attached Proton Test (APT), the Distortionless Enhancement Polarization Transfer (DEPT), correlated spectroscopy (COSY), Heteronuclear Multiple Quantum Coherence Spectroscopy (HMQC), NMR spectra for each compound.

Example 3

Measurement of the neuromuscular blocking activity of GS-53 in rats

[0040] Male Wistar rats were prepared following modifications of the reported methods (Henning, R. Br. J. Pharmacol. 1993, 108, 717-720). The animals were anaesthetized using 25% urethane in water (w/v, 1.25 g/kg i.p). Each animal was laid on its back and fixed to a surgical board and body temperature was maintained by an over-head lamp. An incision was made on the neck, the trachea was located freed and a cannula was inserted to supply artificial ventilation with room air delivered by Parvalux Electric Motors Ltd rodents' respirator (Wallisdown, Bournermouth, England) at a frequency of 90 breaths per minute and a tidal volume of 20 c.c./kg. Furthermore, an incision was made to remove the skin covering the tibialis and its neighbor muscles on the right leg. The membrane covering the tibialis muscle was removed and its tendon freed from the Knob in the middle of the foot. The tendon was tied with a strong thread, passed through a bully, attached to a force displacement transducer (10-100 g, Narco-Biosystems, Myograph F 2000; USA) which was connected to a Narco Physiograph via Universal Coupler Type 7173. An incision was made on the lateral right side of the animal just above the site of the sciatic nerve supplying the tibialis muscle. The sciatic nerve was freed and the nerve was secured between a platinum electrodes. A strong tie was made on the portion of the nerve nearer to the spinal cord to prevent a generalized electrical stimulation. The electrodes were attached to an electric stimulator (Science and Research Instruments, Ltd, Kent, U.K.). Both of the muscle and the nerve were covered with paraffin oil at 37°C to prevent dryness of the tissues. The Tibialis muscle twitches were induced using the following parameters: supramaximal voltage of 40 volts, at a frequency of 0.3 Hz and 0.5 msec duration. When tetanus was performed the frequency was increased to 30 Hz and the speed of recording was increased from 0.25 cm/sec to 1 cm/sec and stimulation was performed for 5-10 sec. The calibration system built in the transducer was used to measure the tension of the muscle. Changes in the muscle tension (twitches) were expressed as percentage change from the pre-drug values.

[0041] **GS-53**, standards or vehicles were administered intraperitoneally dissolved in dimethyl sulfoxide, or water as appropriate in various doses 1-100 mg/kg. The effect of each dose on the twitch response was calculated as a percentage of the pre-drug amplitude. The ED_{50} was calculated for **GS-53**. Also the dose that produced 90% of twitches depression was calculated. The response of the muscle to titanic stimulation was tested before administration of **GS-53** and following 90% inhibition of the twitches. To investigate the reversibility of any block, the anticholinesterase - Physostigmine was injected in a single bolus injection of 100 μg/kg. The onset time of reversal and the percentage of reversal were monitored and calculated, respectively. The duration of each block was monitored. In all animals rectal temperature was maintained at 37±1°C. The duration of action of **GS-53** was 2.3 times that of Atracurium, and its blockade was completely reversed following administration of Physostigmine (Table 1).

Table 1: Summary of the Effects of **GS-53**, Atracurium and Succinylcholine on the rat tibialis electrically-induced twitches together with the effects of tetanus and physostigmine at a dose of 100 mg/kg i.p. (N = 4-8 animals).

*Parameter	GS-53	Atracurium	Succinylcholine
ED_{50} (mmole/kg i.p.)	0.15 (48.6 mg)	0.016	0.03
Time of onset of Block (min)	3 ± 0.9	5 ± 0.8	2 ± 0.1
Time of 50% Block (min)	7 ± 0.6	8 ± 0.2	3 ± 0.1
Time of 90% Block (min)	10 ± 3	12 ± 1.3	5 ± 0.2
Effect of Tetanus (30 Hz)	Non-Maintained	Non-Maintained	Well-Maintained
Effect of Physostigmine	100% Reversal	100% Reversal	No Reversal
Time of onset of Reversal (min)	1.0	2.0	-
Time for 100% Reversal (min)	6 ± 1.2	3 ± 0.4	-
Duration of the Block (min)	70 ± 12	30 ± 6	20 ± 1.8

*The results reported were the mean±S.E., N = number of animals used. Significant differences between the various treatments were performed using paired or un-paired t-test. P values < 0.05 were considered significant.

Table 2: Effects of **GS-53**, Atracurium and Succinyl choline in chicks

Parameter	GS-53	Atracurium	Succinyl choline
ED_{50} (mmole/kg i.p.)	0.15 (48.6 mg)	0.016	0.03
Onset of head drop (min)	0.25 ± 0.1	5 ± 0.8	-
Onset of muscle relaxation (min)	1 ± 0.08	10 ± 0.8	0.5 ± 0.02
Type of neuromuscular paralysis	Flaccid	Flaccid	Spastic
Mechanism of action	Competitive	Competitive	Depolarizing

*The results reported were the mean±S.E., N = number of animals used. Significant differences between the various treatments were performed using paired or un-paired t-test. P values < 0.05 were considered significant.

Example 4

Measurement of the neuromuscular blocking activity of GS-53 in chicks

[0042] The effects of GS-53 and the standard drugs Succinylcholine and Atracurium were administered to 4-day old chicks to confirm the mode of the neuromuscular blockade (Buttle, G. et al, J Pharm. Pharmacol. 1949, 1, 991-992; Thesleff, S. et al, J. Pharmacol. Expt. Ther. 1954, 111, 99-118; ELTahir, K. Guide to Drug Discovery, 2008, Riyadh, KSA). Sub-maximal dose of GS-53 and the standards as revealed in the rats studies were administered (i.p) to the chicks and the type of paralysis was noted. Intraperitoneal administration of GS-53 in single dose of 0.15 mmoles/kg, induced flaccid paralysis of the chicks within one minute. The paralysis started with head movement and drop; then complete flaccid paralysis occurred. The onset of head drop was 15 sec whereas the flaccid paralysis was one minute following administration. Atracurium and Succinylcholine were used as positive controls. The mechanism of action GS-53 seemed to be similar but with more rapid onset of action than that of Atracurium; and almost similar to that of Succinylcholine (Table 2).

Example 5

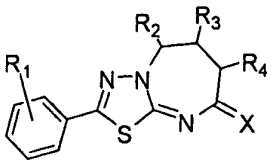
Determination of the Lethal Dose (LD_{50}) of GS-53

[0043] Male mice were divided into various groups and GS-53 was administered in various doses ranging from 0.1-5 mmole/kg, intraperitoneal. Following treatments, the animals were observed for up to 6 hours continuously and were

then kept under observation for 72 hours. All behavioral changes and death during the observation periods were recorded. The percentage of death at each dose level was then calculated, converted to probits and the LD_{50} values were calculated as outlined by (Ghosh, M., Fundamentals of Experimental Pharmacology, Scientific Book Agency, Calcutta. 1984, pp 153-158, 187-189). the calculated LD_{50} of (*E*)-2-(4-bromophcnyl)-6,7-dihydro-[1,3,4]thiadiazolo[3,2-*a*][1,3]diazepin-8 (5*H*)-one (GS-53, Example 2) was found to be 195.0 mg/kg with 95% confidence limits of 185.25-204.75 mg/kg.

Claims

1. Compound according to formula 1



wherein R_1, R_2, R_3 and R_4 are each independently selected from the group consisting of hydrogen, halogen, and, C_1-C_{20}-alkyl, C_1-C_{20}-haloalkyl, C_1-C_{20}-alkoxy, C_1-C_{20}-haloalkoxy, aryl, heteroaryl, mercapto, alkylthio, amino, and alkylamino, optionally substituted by halogen, amino, C_1-C_{20}-alkyl, C_1-C_{20}-haloalkyl, C_1-C_{20}-alkoxy, C_1-C_{20}-haloalkoxy, mercapto, alkylthio, alkylamino, arylthio, heteroarylthio, arylamino or heteroarylamino, or wherein R_3 and R_4, are a member of an, alicyclic, aryl or heteroaryl ring system optionally substituted by halogen, amino, C_1-C_{20}-alkyl, C_1-C_{20}-haloalkyl, C_1-C_{20}-alkoxy, C_1-C_{20}-haloalkoxy, mercapto, alkylthio, alkylamino, arylthio, heteroarylthio, arylamino or heteroarylamino, and wherein X is selected from O or S,
or wherein the compound is present in form of its addition salts.

2. Compound according to claim 1, wherein R_2 is selected from the group consisting of hydrogen, mercapto, and C_1-C_{20}-alkyl.

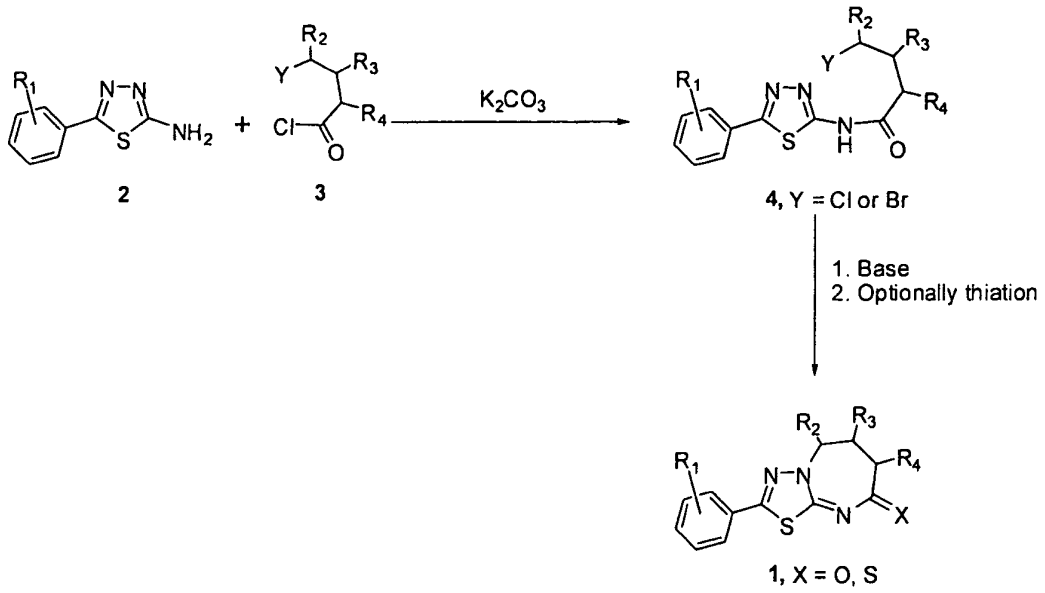
3. Compound according to claim 1 or 2, wherein R_3 is hydrogen or is taken together with R_4 to form an, alicyclic, aryl or heteroaryl ring system, optionally substituted by halogen, amino, C_1-C_{20}-alkyl, C_1-C_{20}-haloalkyl, C_1-C_{20}-alkoxy, C_1-C_{20}-haloalkoxy, mercapto, alkylthio, alkylamino, arylthio, heteroarylthio, arylamino or heteroarylamino,.

4. Compound according to claim 1, wherein R_1, R_2, R_3 and R_4 are independently selected from aryl or heteroaryl.

5. Compound according to any of the preceding claims, wherein the compound is present in form of its addition salt of hydrochloride, hydrobromide, phosphate, nitrate, acetate, malate, succinate, fumarate, tartrate, salicylate, sorbate, lactate, p-toluene sulphate, or naphthalene-1,5-disulfonate salts.

6. Method for preparing a compound according to any of the claims 1-5, comprising reacting a compound according to formula 2 with the compound according to formula 3 to prepare a compound according to formula 4, and reacting the compound of formula 4 to result in the compound of formula 1, as given scheme 1 as follows:

Scheme 1



7. Method according to claim 6, wherein the reaction of compound 2 and compound 3 is in the presence of potassium carbonate in a solvent.

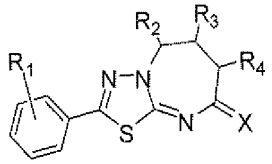
8. Method according to claim 6 or 7, wherein compound 4 is cyclized in the presence of secondary amines in a solvent.

9. Pharmaceutical composition comprising at least one compound according to any of the claims 1-5 and a pharmaceutically acceptable carrier or excipient.

10. Compound according to any of the claims 1 to 5 or the pharmaceutical composition according to claim 9 for use as neuromuscular blocker or skeletal muscle relaxant.

Patentansprüche

1. Verbindung nach Formel 1



wobei R_1, R_2, R_3 und R_4 jeweils unabhängig ausgewählt sind aus der Gruppe bestehend aus Wasserstoff, Halogen und C_1-C_{20}-Alkyl, C_1-C_{20}-Halogenalkyl, C_1-C_{20}-Alkoxy, C_1-C_{20}-Halogenalkoxy, Aryl, Heteroaryl, Mercapto, Alkylthio, Amino und Alkylamino, wahlweise substituiert mit Halogen, Amino, C_1-C_{20}-Allcyl, C_1-C_{20}-Halogenalkyl, C_1-C_{20}-Alkoxy, C_1-C_{20}-Halogenalkoxy, Mercapto, Alkylthio, Alkylamino, Arylthio, Heteroarylthio, Arylamino oder Heteroarylamino, oder wobei R_3 und R_4 Teil eines alizyklischen, Aryl- oder Heteroarylringsystems sind, wahlweise substituiert mit Halogen, Amino, C_1-C_{20}-Allcyl, C_1-C_{20}-Halogenalkyl, C_1-C_{20}-Alkoxy, C_1-C_{20}-Halogenalkoxy, Mercapto, Alkylthio, Alkylamino, Arylthio, Heteroarylthio, Arylamino oder Heteroarylamino, und wobei X ausgewählt ist aus O oder S,
oder wobei die Verbindung in Form ihres Additionssalzes vorliegt.

2. Verbindung nach Anspruch 1, wobei R_2 ausgewählt ist aus der Gruppe bestehend aus Wasserstoff, Mercapto und C_1-C_{20}-Alkyl.

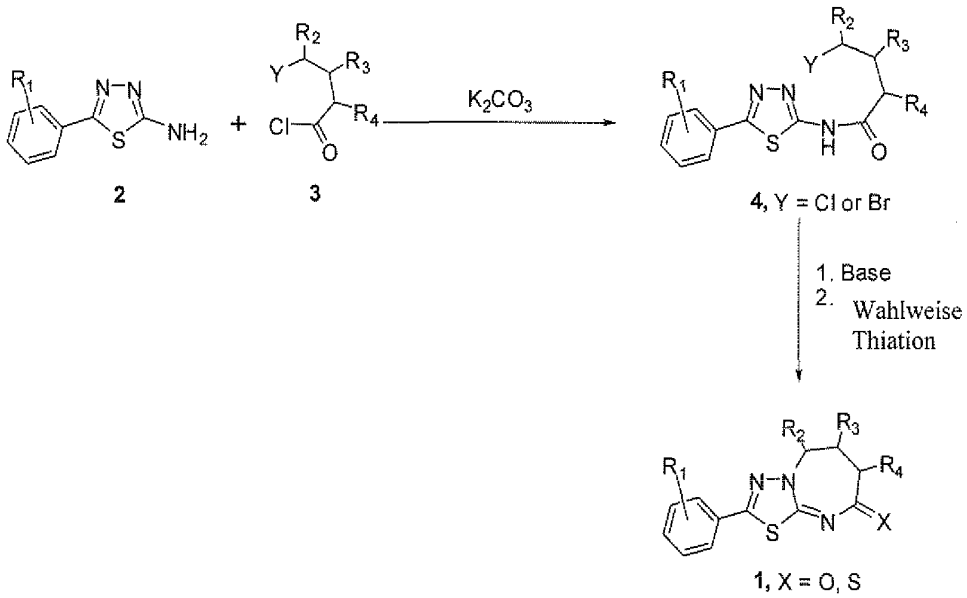
3. Verbindung nach Anspruch 1 oder 2, wobei R_3 Wasserstoff ist oder zusammen mit R_4 genommen wird, um ein alizyklisches, Aryl- oder Heteroarylringsystem zu bilden, wahlweise substituiert mit Halogen, Amino, C_1-C_{20}-Alkyl, C_1-C_{20}-Halogenalkyl, C_1-C_{20}-Alkoxy, C_1-C_{20}-Halogenalkoxy, Mercapto, Alkylthio, Alkylamino, Arylthio, Heteroarylthio, Arylamino oder Heteroarylamino.

4. Verbindung nach Anspruch 1, wobei R_1, R_2, R_3 und R_4 unabhängig ausgewählt sind aus Aryl oder Heteroaryl.

5. Verbindung nach einem der vorangegangenen Ansprüche, wobei die Verbindung in Form ihres Additionssalzes aus Hydrochlorid-, Hydrobromid, Phosphat-, Nitrat-, Acetat-, Malat-, Succinat-, Fumarat-, Tartrat-, Salicylat-, Sorbat-, Lactat-, p-Tuolsulfat- oder Naphthalen-1,5-disulfonat-Salzen vorliegt.

6. Verfahren für die Herstellung einer Verbindung nach einem der Ansprüche 1-5, umfassend Umsetzen einer Verbindung nach Formel 2 mit der Verbindung nach Formel 3, um eine Verbindung nach Formel 4 herzustellen, und Umsetzen der Verbindung der Formel 4, um die Verbindung der Formel 1 zu erhalten, wie laut Schema 1 gegeben:

Schema 1



7. Verfahren nach Anspruch 6, wobei die Reaktion der Verbindung 2 und der Verbindung 3 in der Gegenwart von Kaliumcarbonat in einem Lösungsmittel ist.

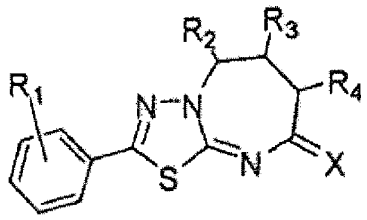
8. Verfahren nach Anspruch 6 oder 7, wobei Verbindung 4 in der Gegenwart sekundärer Amine in einem Lösungsmittel zyklisiert wird.

9. Pharmazeutische Zusammensetzung umfassend mindestens eine Verbindung nach einem der Ansprüche 1-5 und einen pharmazeutischen annehmbaren Träger oder Hilfsstoff.

10. Verbindung nach einem der Ansprüche 1 bis 5 oder pharmazeutische Zusammensetzung nach Anspruch 9 für die Verwendung als neuromuskulärer Blocker oder Skeletalmuskelrelaxans.

Revendications

1. Composé selon la formule 1



dans lequel R_1, R_2, R_3 et R_4 sont choisis chacun indépendamment dans le groupe consistant en un hydrogène, un halogène, et, un alkyle en C_1-C_{20}, un haloalkyle en C_1-C_{20}, un alcoxy en C_1-C_{20}, un haloalcoxy en C_1-C_{20}, un aryle, un hétéroaryle, un mercapto, un alkylthio, un amino, et un alkylamino, optionnellement substitué par un halogène, un amino, un alkyle en C_1-C_{20}, un haloalkyle en C_1-C_{20}, un alcoxy en C_1-C_{20},un haloalcoxy en C_1-C_{20}, un mercapto, un alkylthio, un alkylamino, un arylthio, un hétéroarylthio, un arylamino ou un hétéroarylamino, ou dans lequel R_3 et R_4, sont un membre d'un système cyclique, alicyclique, aryle ou hétéroaryle optionnement substitué par un halogène, un amino, un alkyle en C_1-C_{20}, un haloalkyl en C_1-C_{20}, un alcoxy en C_1-C_{20}, un haloalcoxy en C_1-C_{20}, un mercapto, un alkylthio, un alkylamino, un arylthio, un hétéroarylthio, un arylamino ou un hétéroarylamino, et dans lequel X est choisi parmi O ou S,
ou dans lequel le composé est présent sous forme de ses sels d'addition.

2. Composé selon la revendication 1, dans lequel R_2 est choisi dans le groupe consistant en un hydrogène, un mercapto, et un alkyle en C_1-C_{20}.

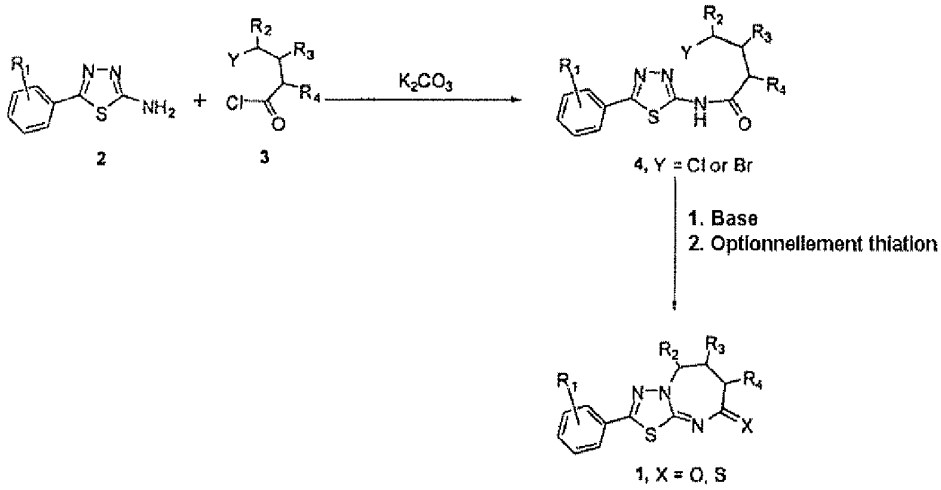
3. Composé selon la revendication 1 ou 2, dans lequel R_3 est un hydrogène ou est pris conjointement avec R_4 pour former un système cyclique, alicyclique, aryle ou hétéroaryle, optionellement substitué par un halogène, un amino, un alkyle en C_1-C_{20}, un haloalkyle en C_1-C_{20}, un alcoxy en C_1-C_{20}, un haloalcoxy en C_1-C_{20}, un mercapto, un alkylthio, un alkylamino, un arylthio, un hétéroarylthio, un arylamino ou un hétéroarylamino.

4. Composé selon la revendication 1, dans lequel R_1, R_2, R_3 et R_4 sont indépendamment choisis parmi un aryle ou un hétéroaryle.

5. Composé selon l'une quelconque des revendications précédentes, dans lequel le composé est présent sous forme de son sel d'addition de chlorhydrate, bromhydrate, phosphate, nitrate, acétate, malate, succinate, fumarate, tartrate, salicylate, sorbate, lactate, sulfate de p-toluène, ou des sels de naphtalène-1,5-disulfonate.

6. Procédé de préparation d'un composé selon l'une quelconque des revendications 1-5, comprenant la réaction d'un composé selon la formule 2 avec le composé selon la formule 3 pour préparer un composé selon la formule 4, et en faisant réagir le composé de formule 4 pour donner lieu au composé de formule 1, tel qu'indiqué dans le schéma 1 qui suit:

Schéma 1



7. Procédé selon la revendication 6, dans lequel la réaction du composé 2 et du composé 3 est en présence de carbonate de potassium dans un solvant.

8. Procédé selon la revendication 6 ou 7, dans lequel le composé 4 est cyclisé en présence d'amines secondaires dans un solvant.

9. Composition pharmaceutique comprenant au moins un composé selon l'une quelconque des revendications 1-5 et un support ou excipient pharmaceutiquement acceptable.

10. Composé selon l'une quelconque des revendications 1 à 5 ou la composition pharmaceutique selon la revendication 9 pour son utilisation en tant que bloqueur neuromusculaire ou relaxant musculaire squelettique.

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