USE OF SELECTIVE COX-2 INHIBITORS FOR THE TREATMENT OF URINARY INCONTINENCE

FIELD OF THE INVENTION

The invention relates to compositions and methods for treating neuromuscular dysfunction of the lower urinary tract in mammals, including humans, using selective inhibitors of the cyclooxygenase-2 isozyme (COX-2).

BACKGROUND OF THE INVENTION

In mammals, micturition is a complex process that requires the integrated actions of the bladder, its internal and external sphincters, and the musculature of the pelvic floor. Neurological control over these muscles occurs at three levels- in the bladder wall or sphincters, in the autonomic ganglia of the spinal cord, and in the central nervous system in the pontine micturition center of the brainstem (pons), under the control of the cerebral cortex.

Micturition results from contraction of the bladder detrusor muscle, which consists of interlacing smooth muscle fibers that are under parasympathetic autonomic control from the sacral spinal cord. A simple voiding reflex is formed by sensory nerves for pain, temperature, and distension that run from the bladder to the sacral cord. However, sensory tracts from the bladder also reach the pontine micturition center, resulting in the generation of nerve impulses that normally act at the spinal cord to suppress the sacral spinal reflex arc controlling bladder emptying. As a result, normal micturition is initiated by voluntary suppression of the cortical inhibition of the reflex arc and by relaxation of the muscles of the pelvic floor and the external sphincter. These events are followed by contraction of the detrusor muscle and voiding.

Functional abnormalities of the lower urinary tract, *e.g.*, dysuria, incontinence, and enuresis, are common in the general population. Dysuria includes urinary frequency, nocturia, and urgency, and may be caused by cystitis, prostatitis or benign prostatic hypertrophy, which affects about 70% of elderly males, or by neurological disorders. Incontinence syndromes include stress incontinence, urgency incontinence, and overflow incontinence. Enuresis refers to the involuntary passage of urine at night or during sleep.

Prior to the present invention, treatment of neuromuscular disorders of the lower urinary tract has

involved administration of compounds that act directly on the bladder muscles, such as flavoxate, a spasmolytic drug that is also active on the pontine micturition center, or anticholinergic compounds such as oxybutynin. The use of α_1-adrenergic receptor antagonists for the treatment of benign prostatic hypertrophy is also common. However, treatments that involve direct inhibition of the pelvic musculature or detrusor muscle may have undesirable side effects, such as incomplete voiding, accommodation reflex paralysis, tachycardia and dry mouth. Thus, it would be preferable to treat neuromuscular disorders of the lower urinary tract with compounds that act via the peripheral or central nervous system, to affect, for example, the sacral spinal reflex arc and/or the inhibitory impulses of the pontine micturition center in a manner that restores normal functioning of the micturition mechanism.

Available evidence indicates that arachidonic acid metabolites produced via the cyclooxygenase (COX) pathway are involved in the physiological regulation of the micturition reflex. Prostaglandin E_1 (PGE_1), PGE_2, PGD_2, $PGF_{2\alpha}$ as well as tromboxane A_2 (TXA_2) have been shown to induce contractile activity of animal and human bladder detrusor, *in vitro* (Andersson *et al.*, Acta Physiol. Scand. 100:165-171, 1977; Borda *et al.*, J. Urol. 129:1250-1253, 1983; Maggi *et al.*, J. Pharmacol. Exp. Ther. 230:500-513, 1984; Gotoh *et al.*, J. Urol. 135:431-437, 1986; and Palea *et al.*, Br. J. Pharmacol. 124:865-872, 1998). PGE_2 and $PGF_{2\alpha}$ also play a role in modulating cholinergic and purinergic contractions generated by electrical stimulation, as demonstrated in isolated rabbit urinary bladder (Downie *et al.*, J. Pharmacol. Exp. Ther. 230:445-449, 1984 and Husted *et al.*, Arch. Intl. Pharmacodyn. 246:84-97, 1980). Furthermore, distension of the bladder wall leads to local production of endogenous prostanoids that modulate the afferent branch of the micturition reflex by lowering the threshold for eliciting voiding contractions (Ghoneim *et al.*, J. Urol. 116:739-743, 1976), thus representing the link between stretching of the detrusor muscle due to bladder filling and activation of capsaicin-sensitive afferents.

Accordingly, indomethacin and flurbiprofen, well known inhibitors of cyclooxygenase, showed favorable effects in double-blind controlled studies in patients with detrusor instability, although they did not completely abolish detrusor overactivity and showed a high incidence of gastrointestinal side effects (Andersson, Drugs 35:477-494, 1988).

Prostaglandins are produced from free arachidonic acid through the catalytic activity of two COX enzymes. COX-1 is considered the constitutive isoform, being expressed in almost all tissues,

whereas COX-2 is considered inducible, since its expression can be triggered typically by inflammatory insults. Several groups, however, have reported constitutive expression of COX-2 in different tissues, including the central nervous system (Yasojima *et al.*, Brain Res. 830:226-236, 1999).

In the brainstem, COX-2-containing neurons were observed in the dorsal raphe nucleus, the nucleus of the brachium of the inferior colliculus, and in the region of the subcoeruleus, suggesting that COX-2 may be involved in the processing and integration of visceral sensory input and in the modulation of autonomic responses (Breder *et al.*, J. Comp. Neurol. 355:296-315, 1995). Furthermore, it has been demonstrated (Yamamoto *et al.*, Brain Res. 739:104-110, 1996) that COX-2 is constitutively expressed in the spinal cord, where it plays an important role in the spinal nociceptive information transmission.

Previous attempts did not establish a role for COX-2 specific antagonists in the treatment of non-inflammatory based neuromuscular dysfunction of the lower urinary tract. Lecci *et al.* (Br. J. Pharm. 130:331-338, 2000) compared the effects of a non-selective COX-1/COX-2 inhibitor, dexketoprofen, and a COX-2 selective inhibitor, NS-398, on urodynamic function, in both inflammation-based and non-inflammation based rat models. Bladder contractions elicited by application of arachidonic acid onto the serosal surface of the urinary bladder were blocked by dexketoprofen, but not by NS-398. Furthermore, although both dexketoprofen and NS-398 normalized bladder reflexes following either surgery or experimentally-induced inflammation of the lower urinary tract, there was no enhanced effect evidenced by the COX-2-specific inhibitor.

Lecci *et al.*, therefore, failed to show any effect of NS-398 in a non-inflammation-based model of bladder function. Nor did Lecci *et al.* demonstrate a selective effect of a COX-2 inhibitor in inflammation-based models of urodynamic function. Nor did Lecci *et al.* demonstrate that selective COX-2 inhibitors are more potent than non-selective COX-1/COX-2 inhibitors at inhibiting the central part of the micturition reflex. Given these results, Lecci *et al.* speculated that, under non-inflammatory conditions, only the blockade of both COX-1 and COX-2 leads to urodynamic changes.

PCT application WO 98/09948 measured the effect *in vitro* of the COX-2 specific inhibitor, nimesulide, on contractility of isolated rat bladder strips stimulated with 40 mM potassium. Nimesulide was ineffective at inhibiting contractility, in that a high concentration (1×10^{-4} M) of

nimesulide achieved only a 42% inhibition. The requirement for such a high concentration suggests that the observed effect was not due neuronal control of micturition, but rather was due to local effect of nimesulide on the bladder dome tissue.

Thus, there remains a need in the art for potent COX-2 specific inhibitors that are effective in treating neuromuscular dysfunction of the lower urinary tract. In particular, there remains a need for COX-2 specific inhibitors that are effective in treating neuromuscular dysfunction by acting upon the micturition reflex.

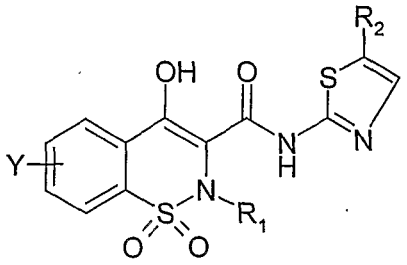
We have tested selective and non-selective (*e.g.,* indomethacin) COX-2 inhibitors for their activity on the lower urinary tract in the rat. Using an animal model that reflects the central mechanisms underlying bladder control to test the effects of specific and non-specific COX-2 inhibitors on bladder function, we have found, unexpectedly, that selective COX-2 inhibitors proved very potent in inhibiting frequency of bladder contraction.

Given these results, selective COX-2 inhibitors can be an effective means to treat urinary tract disorders.

SUMMARY OF THE INVENTION

The invention is based on the finding that selective COX-2 inhibitors are useful in the treatment of neuromuscular dysfunction of the lower urinary tract in mammals. Accordingly the invention provides the use of certain selective COX-2 inhibitors for the preparation of medicaments for the treatment of neuromuscular dysfunction of the lower urinary tract in mammals, including without limitation, dysuria, incontinence and enuresis. The selective COX-2 inhibitors for use in the invention are preferably those of the formulae **I** to **VI** below. All hydrates, solvates, polymorphs and pharmaceutically acceptable salts of the compounds described may be substituted for the compounds themselves. Some of the compounds described contain one or more asymmetric centers and may thus give rise to diastereomers and optical isomers. The compounds may be used in the invention in the form of such diastereomers as well as their racemic and resolved, enantiomerically pure forms. Likewise, some of the compounds described herein contain olefinic double bonds. The compounds may be used in the invention in the form of both E and Z geometric isomers of such compounds.

Compounds I are those having the general formula



(I)

wherein:

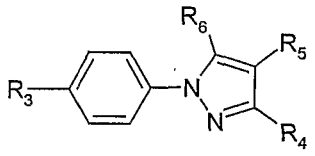
R_1 represents a hydrogen atom or a methyl or ethyl group;

R_2 represents a methyl, ethyl or n-propyl group; and

Y represents a hydrogen, fluorine or chlorine atom or a methyl or methoxy group.

In a preferred embodiment, R_1 and R_2 both represent methyl groups and Y represents a hydrogen atom. This is the compound 1,1-dioxo-4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1,2-benzothiazine-3-carboxamide (Compound A).

Compounds II are those having the general formula



(II)

wherein:

R_3 represents a $NHS(O)_2CH_3$, $S(O)_2CH_3$, $S(O)_2NH_2$, $S(O)(NH)NH_2$, or $S(O)_2NHC(O)(CH_2)_{1\text{-}3}CH_3$ group;

R_4 represents a halogen atom or a haloalkyl, cyano, nitro, aminocarbonyl, N-alkylaminocarbonyl, N,N-dialkylaminocarbonyl, cycloalkylaminocarbonyl, haloaralkyl, aminocarbonylhaloalkyl, alkylsulfonyl or N-alkylaminosulfonyl group;

R_5 represents a hydrogen or halogen atom or an alkyl, thio, alkylthio, haloalkyl, cyano, hydroxyalkyl, alkylsulfonyl or cycloalkyl group; and

R_6 represents an aryl, cycloalkyl, cycloalkenyl or heterocyclic group and is optionally substituted

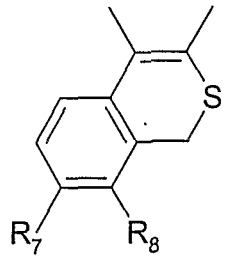
with one or more radicals selected from halogen atoms and alkylthio, alkyl, alkenyl, alkylsulfonyl, cyano, aminocarbonyl, haloalkyl, hydroxy, alkoxy, hydroxyalkyl, haloalkoxy, sulfamyl, amino, heterocyclic, cycloalkylalkyl and nitro groups, or

R_5 and R_6 together with the pyrazole ring atoms to which they are attached form a 6-or 7-membered ring in which the ring atom next to that in the 4-position in the pyrazole ring is a sulfur atom, the 6- or 7-membered ring optionally being fused with a benzene ring which itself may be substituted with one or more halogen atoms and/or C_{1-4} alkyl groups.

The preferred group that R_3 represents is $S(O)_2NH_2$. The preferred haloalkyl that R_4 represents is trifluoromethyl. The preferred aryl group that R_6 represents is phenyl, optionally substituted with a radical selected from halogen atoms and C_{1-4} alkyl, C_{2-4} alkenyl, C_{1-4} haloalkyl, hydroxyl, C_{1-4} alkoxy, hydroxyalkyl, haloalkoxy, and amino groups. All of the foregoing are independent preferences available in all combinations..

Preferred simultaneously are where R_3 is $S(O)_2NH_2$, R_4 is trifluoromethyl, R_5 is hydrogen and R_6 represents phenyl substituted with a methyl, ethyl, methoxy or ethoxy group.

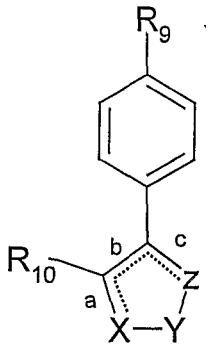
When R_5 and R_6 are taken together as above described, they preferably form a group represented by the structure,



wherein R_7 represents a C_{1-4} lower alkoxy and R_8 represents a halogen atom. Most preferred is where R_7 is methoxy and R_8 is fluorine.

Synthesis of the compounds of formula II are described in WO 95/15316 and WO 96/09304. Additional chemistry relevant to synthesis of compounds of formula II may be found in Reid *et al.* (J. Amer. Chem. Soc. 72:2948-2952, 1950).

Compounds III are those having the general formula



(III)

wherein

when side b is a double bond and sides a and c are single bonds X-Y-Z represents one of the groups

$-CH_2CH_2CH_2-$,

$-C(O)CH_2CH_2-$,

$-CH_2CH_2C(O)-$,

$-CR_{11}(R'_{11})-O-C(O)-$,

$-C(O)-O-CR_{11}(R'_{11})-$,

$-CR_{11}(R'_{11})-NR_{12}-C(O)-$,

$-N=CR_{13}-O-$,

$-O-CR_{13}=N-$ and

$-C(O)-NR_{12}-CR_{11}(R'_{11})-$;

or when sides a and c are double bonds and side b is a single bond X-Y-Z represents one of the groups

$=CH-O-CH=$,

$=CH-CR_{11}(R'_{11})-CH=$,

$=CH-NR_{12}-CH=$,

$=N-O-CR_{11}=$,

$=CH-O-N=$ and

$=N-O-N=$;

R_9 represents a $NHS(O)_2CH_3$, $S(O)_2CH_3$, $S(O)_2NH_2$, $S(O)(NH)NH_2$, or $S(O)_2NHC(O)(CH_2)_{1\text{-}3}CH_3$

group;

R_{10} represents a C_{1-6} alkyl or C_{3-7} cycloalkyl group or an unsubstituted or mono-, di- or tri-substituted phenyl or naphthyl group, each substituent for the phenyl or naphthyl group being a halogen atom and/or a C_{1-6} alkyl, C_{1-6} alkoxy, cyano or trifluoromethyl group;

each of R_{11} and R'_{11} independently represents a hydrogen atom or a C_{1-6} alkyl group;

R_{12} represents a hydrogen atom or a trifluoromethyl, C_{1-6}alkyl, hydroxy or C_{1-6} alkoxy group; and

R_{13} represents a hydrogen atom or a trifluoromethyl or C_{1-6}alkyl group.

The following are independent preferences:

Preferred groups that R_9 represents are $S(O)_2CH_3$, $S(O)_2NH_2$ or $S(O)_2NHC(O)(CH_2)_{1-3}CH_3$.

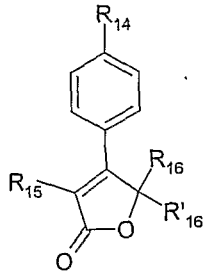
The preferred group that R_{10} represents is a mono-, di-, or tri- substituted phenyl group. More preferred is where R_{10} is a mono-, di-, or trifluorophenyl group.

Preferred groups that R_{11} and R'_{11} may represent independently are hydrogen or methyl.

Preferred groups that R_{12} represents are hydrogen, trifluoromethyl, methyl, ethyl, hydroxy, methoxy, or ethoxy.

Preferred alkyl groups that R_{13} represents are methyl or ethyl.

One preferred subgroup of compounds within the embodiment of formula **III** are compounds of formula **IV(i)**



IV(i)

or pharmaceutically acceptable salts thereof wherein:

R_{14} represents a group of the formula $S(O)_2CH_3$ or $S(O)_2NH_2$;

R_{15} represents a C_{1-6} alkyl, C_{3-7} cycloalkyl group or an unsubsituted, mono-or di-substituted phenyl group, wherein each substituent is selected from the group consisting of halogen atom, C_{1-6} alkyl,

C_{1-6} alkoxy, cyano, and trifluoromethyl; and

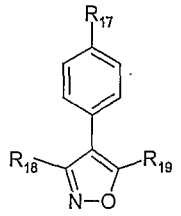
each of R_{16} and R'_{16} independently represents a hydrogen atom or a C_{1-6} alkyl group.

Preferred alkyl groups that R_{15} may represent are methyl or ethyl. Preferred phenyl groups that R_{15} may represent are phenyl groups, optionally substituted with 1 or 2 substituents selected from the group consisting of halogen atom, methyl, ethyl, methoxy, ethoxy, and trifluoromethyl. More preferred, R_{15} may represent a phenyl group, optionally substituted with 1 or 2 halogen atoms. Most preferably, R_{15} may represent a phenyl group, optionally substituted with 1 or 2 fluorine atoms.

Preferred alkyl groups that each of R_{16} and R'_{16} independently represent are methyl or ethyl groups.

More preferred is where each of R_{16} and R'_{16} are hydrogen atoms.

Another preferred subgroup of compounds within the embodiment of formula **III** are compounds of formula **IV(ii)**



IV(ii)

or pharmaceutically acceptable salts thereof wherein:

R_{17} represents $NHS(O)_2CH_3$, $S(O)_2CH_3$, $S(O)_2NH_2$, $S(O)(NH)NH_2$, or $S(O)_2NHC(O)(CH_2)_{1-3}CH_3$,

R_{18} represents a C_{1-6} alkyl, C_{3-7} cycloalkyl group or an unsubsituted, mono-or di-substituted phenyl group, wherein each substituent is selected from the group consisting of halogen atom, C_{1-6} alkyl, C_{1-6} alkoxy, cyano, and trifluoromethyl; and

R_{19} represents a hydrogen atom or a C_{1-6} alkyl group.

Preferred groups that R_{17} represents are $S(O)_2NH_2$ or $S(O)_2NHC(O)CH_2CH_3$.

Preferred alkyl groups that R_{18} may represent are methyl or ethyl. Preferred phenyl groups that R_{18} may represent are phenyl groups, optionally substituted with 1 or 2 substituents selected from the group consisting of halogen atom, methyl, ethyl, methoxy, ethoxy, and trifluoromethyl. More

preferred, R_{18} may represent a phenyl group, optionally substituted with 1 or 2 halogen atoms. Still more preferably, R_{18} may represent a phenyl group, optionally substituted with 1 or 2 fluorine atoms. Most preferably, R_{18} represents an unsubstituted phenyl group.

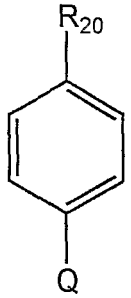
Preferred alkyl groups that each of R_{19} represents are methyl or ethyl groups. Most preferably, $R_{19}$is a methyl group.

A preferred embodiment is where, simultaneously, R_{17} is $S(O)_2NH_2$, R_{18} is an unsubstituted phenyl group and R_{19} is a methyl group.

Another preferred embodiment is where, simultaneously, R_{17} is $S(O)_2NHC(O)CH_2CH_3$, R_{18} is an unsubstituted phenyl group and R_{19} is a methyl group.

The synthesis of compounds of formula **III, IV(i) and IV(ii)** are described in US Pat. No. 5,474,995 and 5,633,272 and in international publication WO 97/38986, respectively. Other general methods used in synthesizing compounds of formula III, IV(i) and IV(ii) can be found in Weissenfels (Z. Chem. 6:471, 1966); Weissenfels (Z. Chem. 13:57, 1973); Kharash (J. Amer. Chem. Soc. 73:3240, 1951); Weinreb (Tet. Lett. 4171, 1977); Girard (J. Org. Chem. 48:3220, 1983); Lau (J. Org. Chem. 51:3038, 1986); Brederick *et al.* (Chem. Ber. p. 88, 1953); Friedman *et al.* (J. Org. Chem. 30:854, 1965); Dimroth *et al.* (Ber. 56:2602, 1956); Dimroth *et al.* (Ann. 634:102, 1961); Schulze *et al.* (Helv. Chimica Acta 74:1059, 1991); Mohammad Aslam *et al.* (J. Org. Chem. 56:5955-5958, 1991); Aliev *et al.* (Sulfur Lett. 12:123-132, 1991); Rutsch *et al.* (Org. Coat. 8:175-195, 1986); Huang *et al.* (Tetrahedron Letts. 35:7204, 1994) and U.S. Pat. No. 4,321,118.

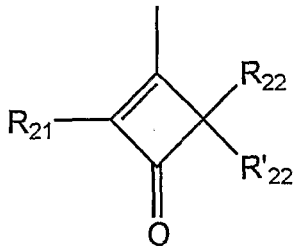
Compounds V are those having the general formula



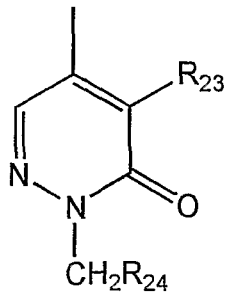
V

wherein R_{20} represents a $NHS(O)_2CH_3$, $S(O)_2CH_3$, $S(O)_2NH_2$, $S(O)(NH)NH_2$ or $S(O)_2NHC(O)(CH_2)_{1\text{-}3}CH_3$ group and Q represents one of the groups of the formulae Q(i) to Q(v) below

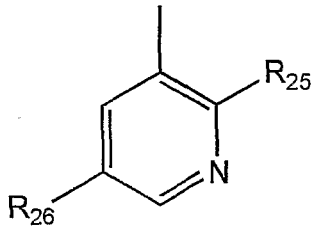
(i)



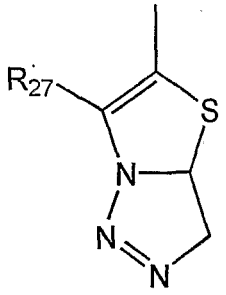
(ii)



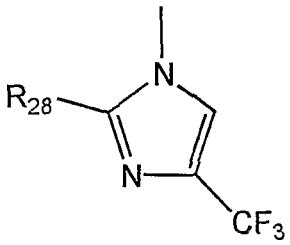
(iii)



(iv)



(v)



wherein R_{21} represents a C_{1-6} alkyl or C_{3-7} cycloalkyl group or an unsubstituted or mono-, di- or tri-substituted phenyl or naphthyl group, each substituent for the phenyl or naphthyl group being a halogen atom and/or a C_{1-6} alkyl, C_{1-6} alkoxy, cyano or trifluoromethyl group;

each of R_{22} and R'_{22} independently represents a hydrogen atom or a C_{1-6} alkyl group;

R_{23} represents a hydrogen atom or a trifluoromethyl, C_{1-6} alkyl, hydroxy, C_{1-6} branched or unbranched alkoxy group;

R_{24} represents a C_{1-6} alkyl or C_{3-7} cycloalkyl group or an unsubstituted or mono-, di- or tri-substituted phenyl group, each substituent for the phenyl group being a halogen atom and/or a C_{1-6} alkyl, C_{1-6} alkoxy, cyano or trifluoromethyl group;

R_{25} represents a C_{1-6} alkyl or C_{3-7} cycloalkyl group or an unsubstituted or mono-, di- or tri-substituted aryl or heteroaryl group, each substituent for the aryl or heteroaryl group being a halogen atom and/or a C_{1-6} alkyl, C_{1-6} alkoxy, cyano or trifluoromethyl group;

R_{26} represents a hydrogen or halogen atom or a C_{1-6} alkyl or C_{1-6} alkoxy group;

R_{27} represents a C_{1-6} alkyl or C_{3-7} cycloalkyl group or an unsubstituted or mono-, di- or tri-substituted phenyl group, each substituent for the phenyl group being a halogen atom and/or a C_{1-6} alkyl, C_{1-6} alkoxy, cyano or trifluoromethyl group; and

R_{28} represents an unsubstituted or mono-, di-, or tri-substituted heteroaryl group.

The following are independent preferences:

Preferred groups that R_{20} may represent are $S(O)_2CH_3$ or $S(O)_2NH_2$.

Preferred groups that R_{21} represents are phenyl, optionally substituted with 1-2 substituents chosen independently from the group consisting of halogen atom, methyl, ethyl, methoxy, ethoxy, or trifluoromethyl group. More preferred is where R_{21} represents a phenyl group, optionally substituted with 1-2 halogen atoms. Most preferably is where R_{21} represents a phenyl group, optionally substituted with 1-2 fluorine atoms. Preferred alkyl groups that R_{22} and R'_{22} represent independently are methyl and ethyl. In a preferred embodiment, R_{21} represents a phenyl group and each of R_{22} and R'_{22} is a methyl group.

Preferred alkyl groups that R_{23} represents are methyl and ethyl. Preferred alkoxy groups that R_{23} represents are methoxy, ethoxy, n-propoxy, and 2-propoxy. Preferred phenyl groups that R_{24} represents are unsubstituted, mono-, di- or tri-substituted phenyl, wherein each substituent is selected from the group consisting of halogen atom, C_{1-6} alkyl, C_{1-6} alkoxy, cyano and trifluoromethyl. More

preferred is where R_{24} represents unsubstituted, mono- or di-substituted phenyl, wherein the substituents are halogen atoms. In a preferred aspect, R_{23} is 2-propoxy and R_{24} is phenyl.

A preferred group that R_{25} represents is heteroaryl, optionally mono- or di-substituted with halogen, C_{1-4} alkyl or C_{1-4} alkoxy groups. More preferred is where the heteroaryl group that R_{25} represents is pyridinyl, optionally substituted with a C_{1-4} alkyl group. Most preferred is when R_{25} represents a 2-methyl-5-pyridinyl group.

The preferred group that R_{26} represents is halogen. More preferred is where R_{26} is chlorine.

The preferred group that R_{27} represents is phenyl, optionally substituted with 1-3 substituents each selected from the group consisting of halogen atom, C_{1-6} alkoxy and C_{1-6} alkyl. More preferred is where R_{27} represents phenyl.

Preferred heteroaryl groups that R_{28} represents are 3-pyridyl and 4-thiazolyl, optionally substituted with C_{1-4} alkyl group. More preferred is where R_{28} is selected from the group consisting of 3-(2-methylpyridinyl) and 4-(2-methylthiazolyl).

Preferred compounds V include

3-(4-methylsulfonylphenyl)-2-(2-methyl-5-pyridyl)-5-chloro-pyridine,

1-(4-methylsulfonylphenyl)-2-(3-pyridyl)-4-trifluoromethyl-imidazole,

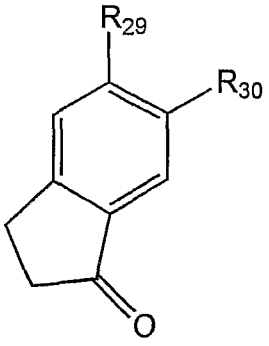
1-(4-sulfamoylphenyl)-2-(2-methyl-3-pyridyl)-4-trifluoromethyl-imidazole, and

1-(4-sulfamoylphenyl)-2-(2-methyl-4-thiazolyl)-4-trifluoromethyl-imidazole.

Syntheses of these compounds are described in WO 97/36863, WO 99/10331, Friessen *et al.* (Bioorgan. and Medic. Chem. Letts. 8:2777-2782, 1998), US Pat. No. 5,552,422 and Khanna*et al.* (J. Med. Chem. 43:3168-3185, 2000).

Compounds VI are those having the general formula

VI



wherein R_{29} represents a $NHS(O)_2CH_3$, $S(O)_2CH_3$, $S(O)_2NH_2$, $S(O)(NH)NH_2$ or $S(O)_2NHC(O)(CH_2)_{1-3}CH_3$ group, and

R_{30} represents a C_{1-6} alkyl or C_{3-7} cycloalkyl group or an unsubstituted or mono-, di- or tri-substituted phenyl or phenylthio group, each substituent for the phenyl or phenylthio group being a halogen atom and/or a C_{1-6} alkyl, C_{1-6} alkoxy group, cyano or trifluoromethyl group.

The following are independent preferences:

The preferred groups that R_{29} represents are $NHS(O)_2CH_3$, $S(O)_2CH_3$ or $S(O)_2NH_2$. The preferred groups that R_{30} represents are mono-, di- or tri-substituted phenyl or phenylthio groups, wherein each substituent is selected from the group consisting of halogen atom, C_{1-6} alkoxy, and C_{1-6} alkyl. More preferred is where R_{30} represents a phenylthio group, wherein the phenyl moiety is optionally substituted with 1-3 halogen atoms. Also preferred is where R_{30} represents a difluorophenylthio group. In a most preferred embodiment, R_{29} represents $NHS(O)_2CH_3$ and R_{30} represents 2,4-difluorophenylthio.

Syntheses of compounds of formula **VI** are described in international publication WO 94/13635. Additional relevant chemistry is described in US Pat. No. 4,375,479.

In another aspect, the invention provides the use for the preparation of a medicament for the treatment of neuromuscular dysfunction of the lower urinary tract of a compound that significantly inhibits the COX-2 isozyme and which exhibits an inhibitory potency for the COX-2 isozyme that is at least 10-fold greater, and preferably at least 100-fold greater than the inhibitory potency said compound exhibits for the COX-1 isozyme.

Systems for ascertaining whether these criteria are met are described below.

Preferably, compounds to be used in practicing the invention should inhibit COX-2 isozyme with an IC_{50} of from about 0.1 to about 100 nM in the models given in Examples 1 and 2.

Administration of the selective COX-2 inhibitors, their stereoisomers, pharmaceutically acceptable salts, hydrates or solvates may be achieved by any effective route, including oral, enteral, intravenous, intramuscular, subcutaneous, transmucosal, and by inhalation routes. Preferable means of administration are via an oral or transdermal route.

Also provided are pharmaceutical formulations comprising the compounds described above in conjunction with pharmaceutically acceptable carriers and/or excipients.

In yet another aspect, the invention provides a method for identifying a compound useful for treating

neuromuscular dysfunction of the lower urinary tract. The method is carried out using the steps of:

(a) individually measuring the inhibitory effects of test compounds on COX-2 and COX-1 isozymes; and

(b) identifying those test compounds that

(1) inhibit the COX-2 isozyme with an IC_{50} at or below concentrations of 10^{-7} M and

(2) inhibit the COX-1 isozyme with an IC_{50} that is at least about 10-fold higher than the IC_{50} the compound exhibits for the COX-2 isozyme.

Preferably, the activity of compounds identified in step (b) above is confirmed by evaluating the inhibition of volume-induced rhythmic bladder voiding contractions in anaesthetized rats (see Example 3).

DETAILED DESCRIPTION OF THE INVENTION:

All patents and other publications and cited herein are hereby incorporated by reference in their entirety. In the case of inconsistencies in definitions, the present description will control.

The invention provides methods and compositions for treatment of neuromuscular dysfunction of the lower urinary tract, particularly those involving micturition (urination) disorder, such as dysuria, incontinence, and enuresis. The methods involve administering to affected individuals selective COX-2 inhibitors for a sufficient time and in an amount effective for lessening or ameliorating at least one symptom of the micturition disorder. Such symptoms include but are not limited to filling symptoms, urgency, incontinence and nocturia, as well as voiding problems such as weak stream, hesitance, intermittency, incomplete bladder emptying and abdominal straining.

The term "treatment" is defined as the prevention, lessening, or amelioration of at least one of the foregoing symptoms of neuromuscular dysfunction of the lower urinary tract.

Efficacy of treatment may be determined by any method known in the art. Such methods include but are not limited determining voiding volumes, frequency of urination, and frequency and strength of bladder contractions in individuals with neuromuscular dysfunction of the lower urinary tract; or and interviewing such individuals to determine if they have experienced the amelioration of any such symptom. Other measures of efficacy include a measurable reduction, most preferably a clinically relevant reduction, of urine leakage related to feelings of urgency, urine leakage related to physical activity, coughing or sneezing, leakage of small amounts of urine (drops), difficulty in bladder

emptying, general leakage not related to urgency or activity, nighttime urination, bedwetting, a feeling of incomplete bladder emptying, etc.

The use of patient-questionnaires and scales to measure symptom severity is widely accepted, complementing objective clinical measures and having the advantage of being inexpensive and potentially self-administered. Female and male lower urinary tract questionnaires have been developed which provide a method of measuring symptom severity and life quality in a reproducible and valid fashion as well as allowing an in-depth assessment of specific lower urinary tract symptoms. Sum of scores collected for the questions included in the questionnaires are highly correlated with subjects' ratings of the magnitude of their urinary problems, and are very sensitive to changes induced by treatment (Barry *et al.*, J. Urol., 148:1549-1557, 1992; Jackson *et al.*, Brit. J. Urol., 77:805-812, 1996).

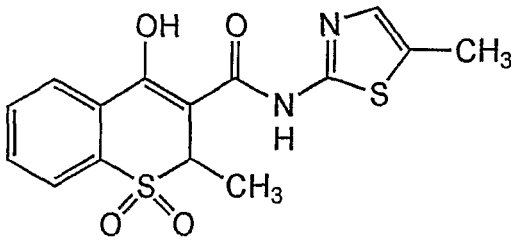
Selective COX-2 inhibitors suitable for use in practicing the invention include without limitation those compounds having one or more of the following properties:

(1) ***Significant COX-2 inhibitory activity*:** Useful compounds preferably exhibit inhibitory potency (IC_{50}) between 100 and 0.1 nM. Without limiting the present disclosure, as described in more detail below, potency may be measured by determining the IC_{50} of compounds *in vivo* or *in vitro*, including cell extracts or fractions of extracts. Inhibitory potency may also be determined using, as non-limiting examples, native or recombinant COX-2 enzymes, enzymes that are expressed constitutively or that have been induced, and enzymes that have expressed in native or non-native species and/or cell types.

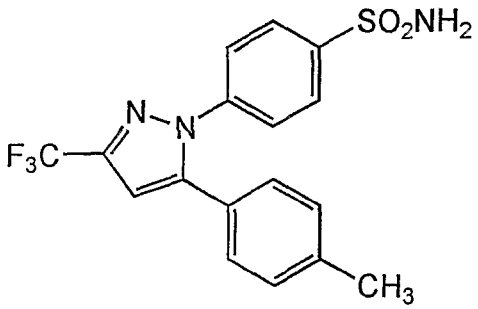
(2) ***Selectivity***: Preferred compounds exhibit at least about 10-fold greater potency in inhibiting the COX-2 isozyme compared to the COX-1 isozyme. More preferred are compounds that exhibit about 100-fold greater potency in inhibiting the COX-2 isozyme compared to the COX-1 isozyme. Accordingly, compounds belonging to this general class are suitable candidates for testing according to the methods taught below.

Compounds that selectively inhibit COX-2 isozyme are therefore candidates for screening to identify compounds useful in treating neuromuscular dysfunction of the lower urinary tract and are exemplified without limitation by:

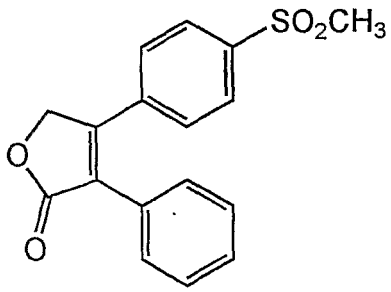
Compound A:



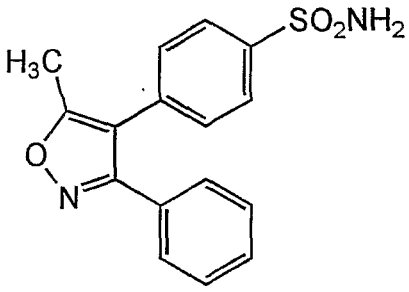
Compound B:



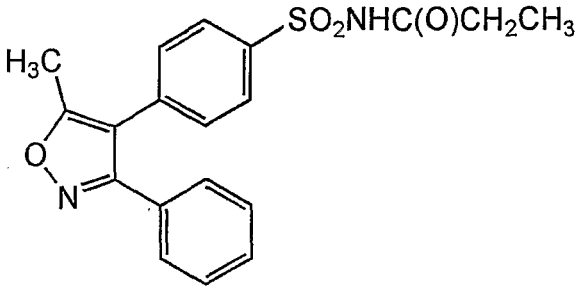
Compound C:



Compound D:



Compound E:



These compounds can be synthesized as described in:
U.S. Patent No. 4,233,299 for A and related compounds;
International publication WO 95/15316 for B and related compounds;
U.S Patent No. 5,474,995 for C and related compounds;
U.S. Patent No. 5,633,272 for D and related compounds; and
International publication WO 97/38986 for E and related compounds.

Screening candidate compounds to identify those compounds that are useful in practicing the invention involves:

1) evaluating their potency and selectivity in inhibiting the COX-2 and COX-1 isozymes; and
2) confirming their pharmacological activity using one or more animal model systems for neuromuscular dysfunction of the lower urinary tract.

To compare the inhibition of COX-1 and COX-2 by various compounds, numerous assays have been developed. The results from these assays are used to calculate a measure of COX-2 selectivity, and compounds are then compared to each other by ranking their COX-2 selectivity.
The commonly used *in vitro* assays for assessing inhibition of COX-1 and COX-2 can be divided into two groups. The first consists of measuring the effect of inhibitors in assays of non-recombinant enzymes purified or partially purified from animal cells, or non-recombinant enzymes that are present in extracts or extract-fractions from animal cells or cell lines. These were the first tests to be developed and are historical in nature. The second type of assay uses human recombinant enzymes that have been expressed in human cell lines or human blood cells, typically platelets and monocytes. These are the current standard tests (Pairet *et al.*, *in* Clinical Significance and Potential of Selective COX-2 Inhibitors, Vane, J. R. and Butting, R. M. (eds.), W. Harwey Press, pp. 1930, 1998).
The COX enzymes used in these assays can be of animal or human origin, they can be native or recombinant, and they can be used either as purified enzymes, microsomal preparations or whole cell assays. In addition, prostaglandin synthesis can be measured either from endogenously released arachidonic acid or from exogenously added arachidonic acid. In assays using recombinant COX-1

and COX-2 enzymes, the expression system used for gene replication and expression also varies. Not all assay systems require a COX-2 inducing agent. For instance, cells may be transfected with recombinant enzymes that are expressed constitutively. In other cell types, however, steps may be required to induce COX-2. In such cases, COX-2 is usually induced with either lipopolysaccharide (LPS) or cytokines, such as interleukin-1 (IL-1) or tumor necrosis factor (Pairet *et al.*, *in* Clinical Significance and Potential of Selective COX-2 Inhibitors, Vane, J. R. and Butting, R. M. (eds.), W. Harwey Press, pp. 19-30, 1998).

In a preferred embodiment, measurement of inhibition of COX isozymes is performed in stably transfected Chinese hamster ovarian (CHO) cells expressing either human COX-1 or COX-2, as previously described (Riendeau *et al.*, Br. J. Pharmacol. 121:105, 1997; Elrich *et al.*, Clin. Pharm. Ther. 65: 336, 1999). Also preferred is where inhibition of COX isozymes is measured on constitutive and inducible forms of human cyclooxygenase (hCOX) cloned and expressed in insect cells (*Spodoptera frugiperda*), utilizing a baculovirus expression system. Expression of COX protein is determined by assessing PG-synthetic capability in homogenates from insect cells (Sf9 cells) incubated for 3 days with COX-1 or COX-2 recombinant baculovirus as previously described (Gierse *et al.*, Biochem. J. 305: 479, 1995).

The inhibitory activity of a test compound can be measured for different isozymes, and the concentration inhibiting the enzyme activity by 50% (IC_{50}) can be calculated using regression analysis, or equivalent computational methods that are well-known in the art (Tallarida *et al.*, Manual of Pharmacologic Calculations. Springer-Verlag, pp. 10-12, 1981.)

As discussed above, compounds useful in practicing the invention inhibit the COX-2 isozyme with an IC_{50} of below 100nM with at least about 10-fold, and preferably about 100-fold, higher potency for the COX-2 isozyme versus the COX-1 isozyme. It will be understood that measurements of inhibitory potency of a particular compound may vary depending upon the source of isozymes, as well as specific assay conditions.

To control for this type of variability, Compound A can be included in all assays as a standardization control. That is, the values of IC_{50} obtained for compound A are compared to the values obtained with tested compounds for each assay. This allows a direct comparison of absolute potency in inhibiting COX isozymes and ratios of inhibitory activities between different assays for the purpose of assessing whether the compound is within the scope of the invention.

A compound is considered to be a "selective" COX-2 inhibitor if it exhibits a selectivity ratio of at least 10 fold, *i.e.*, the IC_{50} for COX-2 is at least 10 fold below the IC_{50} for COX-1.

Once a compound is identified as possessing selectivity for the COX-2 isozyme, its pharmacological activity can be confirmed using one or more animal model systems for neuromuscular dysfunction of the lower urinary tract.

A useful animal model system for measuring such pharmacological activity is, without limitation, volume-induced rhythmic bladder voiding contractions in anesthetized rats. In this method, the urinary bladder is catheterized through the external urethra with a polyethylene tubing filled with physiological saline. The external urethra is then ligated and connected to a pressure recording device. The bladder is then filled with saline until reflex voiding contractions occur, after which the frequency of the voiding contractions is measured for 15 min. Test compounds are then administered intravenously and their effect evaluated for the following 60 min. This method is described in more detail in Example 3 below, and was originally used to validate the predictive qualities of the selective COX-2 inhibitors for the foregoing urinary tract disorders. This model has been validated by the use of different reference standards (Guarneri *et al.*, Pharmacol. Res. 27:173-187, 1993).

Therapeutic Applications

The invention encompasses pharmaceutical formulations comprising those listed above, as well as methods employing these formulations for treating neuromuscular dysfunction of the lower urinary tract such as dysuria, incontinence, and enuresis. Dysuria includes urinary frequency, nocturia, urgency, and difficulty in emptying the bladder, *i.e.*, a suboptimal volume of urine is expelled during micturition. Incontinence syndromes include stress incontinence, urgency incontinence, and overflow incontinence. Enuresis refers to the involuntary passage of urine at night or during sleep. Without wishing to be bound by theory, it is believed that administration of selective COX-2 inhibitors prevents unwanted activity of the bladder afferent neurons and/or inhibits the pontine micturition center controlling micturition. Thus it is contemplated that a wide range of neuromuscular dysfunction of the lower urinary tract can be treated using the compounds of the invention.

An "effective amount" of the compound for treating a urinary disorder is an amount that results in measurable amelioration of at least one symptom or parameter of the disorders described above.

An effective amount for treating the disorder can easily be determined by empirical methods known to those of ordinary skill in the art, such as by establishing a matrix of dosages and frequencies of administration and comparing a group of experimental units or subjects to each point in the matrix. The exact amount to be administered to a patient will vary depending on the state and severity of the disorder and the physical condition of the patient. A measurable amelioration of any symptom or parameter can be determined by a physician skilled in the art or reported by the patient to the physician. It will be understood that any clinically or statistically significant attenuation or amelioration of any symptom or parameter of urinary tract disorders is within the scope of the invention. Clinically significant attenuation or amelioration means perceptible to the patient and/or to the physician or other practitioner.

For example, a single patient may suffer from several symptoms of dysuria simultaneously, such as, for example, urgency and frequency, either or both of which may be reduced using the methods of the invention. In the case of incontinence, any reduction in the frequency or volume of unwanted passage of urine is considered a beneficial effect of the present methods of treatment, and thus, an amelioration of a symptom.

The pharmaceutical compositions of the invention comprise a compound of the invention as an active ingredient or a pharmaceutically acceptable salt, thereof, and may also contain a pharmaceutically acceptable carrier and optionally other therapeutic ingredients. The term "pharmaceutically acceptable salts" refers to salts prepared from pharmaceutically acceptable non-toxic bases including inorganic bases and organic bases. Salts derived from inorganic bases include aluminum, ammonium, calcium, copper, ferric, ferrous, lithium, magnesium, manganic salts, manganous, potassium, sodium, zinc, and the like. Particularly preferred are the ammonium, calcium, magnesium, potassium, and sodium salts. Salts derived from pharmaceutically acceptable organic non-toxic bases include salts of primary, secondary, and tertiary amines, substituted amines including naturally occurring substituted amines, cyclic amines, and basic ion exchange resins, such as arginine, betaine, caffeine, choline, N,N-dibenzylethylenediamine, diethylamine, 2-diethylaminoethanol, 2-dimethylaminoethanol, ethanolamine, ethylenediamine, N-ethylmorpholine, N-ethylpiperidine, glucamine, glucosamine, histidine, hydrabamine, isopropylamine, lysine, methylglucamine, morpholine, piperazine, piperidine, polyamine resins, procaine, purines, theobromine, triethylamine, trimethylamine, tripropylamine, tromethamine, and

the like.

The pharmaceutical compositions containing the active ingredient may be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs. Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents selected from the group consisting of sweetening agents, flavoring agents, coloring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture of tablets. These excipients may be for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch, or alginic acid; binding agents, for example starch, gelatin or acacia, and lubricating agents, for example, magnesium stearate, stearic acid or talc. The tablets may be uncoated or they may be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate may be employed. They may also be coated by the technique described in the US Pat Nos. 4,256,108, 4,166,452 and 4,265,874 to form osmotic therapeutic tablets for control release.

Formulations for oral use may also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredients is mixed with water or an oil medium, for example peanut oil, liquid paraffin, or olive oil.

Aqueous suspensions contain the active material in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydroxy-propylmethycellulose, sodium alginate, polyvinyl-pyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents may be a naturally-occurring phosphatide, for example lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethylene-oxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as

polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives, for example ethyl, or n-propyl, p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose, saccharin or aspartame.

Oily suspensions may be formulated by suspending the active ingredient in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and flavoring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an anti-oxidant such as ascorbic acid.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavoring and coloring agents, may also be present.

The pharmaceutical compositions of the invention may also be in the form of an oil-in-water emulsions. The oily phase may be a vegetable oil, for example olive oil or arachis oil, or a mineral oil, for example liquid paraffin or mixtures of these. Suitable emulsifying agents may be naturally-occurring phosphatides, for example soy bean, lecithin, and esters or partial esters derived from fatty acids and hexitol anhydrides, for example sorbitan monooleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions may also contain sweetening and flavoring agents.

Syrups and elixirs may be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative and flavoring and coloring agents. The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleagenous suspension. This suspension may be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in

1,3-butane diol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

Compounds of the invention may also be administered in the form of a suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials are cocoa butter and polyethylene glycols.

For topical use, creams, ointments, jellies, solutions or suspensions,etc., containing a compound of the invention are employed. (For purposes of this application, topical application shall include mouth washes and gargles.)

Dosage levels of the order of from about 0.01 mg to about 140 mg/kg of body weight per day are useful in the treatment of the above-indicated conditions, or alternatively about 0.5 mg to about 7 g per patient per day. For example, the above-indicated conditions may be effectively treated by the administration of from about 0.01 to about 50 mg of the compound per kilogram of body weight per day, or alternatively about 0.5 mg to about 3.5 g per patient per day. Preferably the agent is administered in a dosage from about 0.1 to about 15 mg of the compound per kilogram of body weight per day and most preferably from about 0.2 to 6 mg of the compound per kilogram of body weight per day.

The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular modeof administration. For example, a formulation intended for the oral administration of humans may contain from 0.5 mg to 5 g of active agent compounded with an appropriate and convenient amount of carder material which may vary from about 5 to about 95 percent of the total composition. Dosage unit forms will generally contain between from about 1 mg to about 500 mg of an active ingredient, for example 5 mg, 12.5 mg, 25 mg, 50 mg, 100 mg, 200 mg, 300 mg, 400 mg or 500 mg.

It will be understood that the pharmaceutical formulations of the invention need not in themselves contain the entire amount of the agent that is effective in treating the disorder, as such effective

amounts can be reached by administration of a plurality of doses of such pharmaceutical formulations.

It will be understood that the specific dose level for any particular patient will depend upon a variety of factors including the age, body weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination, the severity of the particular disease undergoing therapy and the activity of the administered compound.

The methods, tables and examples provided below are intended to more fully describe preferred embodiments of the invention and to demonstrate its advantages and applicability without limiting its scope.

Example 1

Measurement of Inhibition of COX-2 and COX-1 Isozymes in CHO cells

Measurement of inhibition of COX isozymes can be performed in stably transfected Chinese hamster ovarian (CHO) cells expressing either human COX-1 or COX-2, as previously described (Riendeau *et al.*, Br. J. Pharmacol. 121:105, 1997; Elrich*et al.*, Clin. Pharm. Ther. 65: 336, 1999).

Cells (0.3 x 10^6 cells in 200µl) are preincubated in Hank's Balanced Salts Solution (HBSS) containing 15 mM HEPES, pH 7.4, with 3 µl of the test drug or DMSO vehicle for 15 min at 37°C before challenge with arachidonic acid. Cells are challenged for 15 min with an arachidonic acid solution (Stock solution is 100 µM (for COX-2) or 5 µM (for COX-1) arachidonic acid, 10% ethanol in HBSS) to yield final concentrations of 10 µM arachidonic acid in the CHO[COX-2] assay and of 0.5 µM arachidonic acid in the CHO[COX-1] assay. In the absence of exogenous arachidonic acid, levels of PGE_2 in samples from CHO[COX-1] and CHO[COX-2] cells were <80 pg PGE_2 per 10^6 cells. In the presence of 0.5 µM exogenous arachidonic acid, levels of PGE_2 in samples from CHO[COX-1] cells increased to 300 - 2300 pg PGE_2 per 10^6 cells, whereas in the presence of 10 µM exogenous arachidonic acid, levels of PGE_2 in samples from CHO[COX-2] cells increased to 500 - 1400 pg PGE_2 per 10^6 cells.

Cyclooxygenase activity in the absence of test compounds was determined as the difference in PGE_2 levels of cells challenged with arachidonic acid versus the PGE_2 levels in cells mock-challenged with buffer containing ethanol. Each experiment included a set of 8 positive and negative control samples (± arachidonic acid challenge) for cells preincubated in the absence of inhibitor. Compounds were typically tested at 8 concentrations in duplicate with 3 fold serial dilutions in DMSO. Inhibition of

PGE_2 synthesis by test compounds was calculated as a percentage of the activity in the presence of drug versus the activity in the positive control samples.

Example 2

Measurement of Inhibition of COX-2 and COX-1 Isozymes in Sf 9 cells

Constitutive and inducible forms of human cyclooxygenase (hCOX) were cloned and expressed in insect cells (*Spodoptera frugiperda*), utilizing a baculovirus expression system. Expression of COX protein was determined by assessing PG-synthetic capability in homogenates from insect cells (Sf9 cells) incubated for 3 days with COX-1 or COX-2 recombinant baculovirus as previously described (Gierse *et al.*, Biochem. J. 305: 479, 1995).

Cells expressing hCOX-1 or hCOX-2 were homogenized and incubated with arachidonic acid (10μM). COX activity was determined by monitoring PG production. No COX activity was detected in mock-infected Sf9 cells. Test compounds (0.001-100 μM) were preincubated with crude 1% CHAPS homogenates (2-10 μg of protein) for 10 min before addition of arachidonic acid. PGE_2 formed was detected by ELISA, following 10 min incubation.

As shown in Table 1 below, compounds A to C were potent and selective inhibitors of the COX-2 isozyme, but showed undetectable or markedly reduced inhibitory activity of the COX-1 isozyme. Indomethacin was confirmed as a non selective COX inhibitor.

Table 1

Inhibition of COX-2 and COX-1 isozymes

transfected in different cells by tested compounds

Data represent the IC_{50} (concentration inhibiting enzymatic activity by 50%) values (μM) taken from the cited literature.

Tested Compound	COX-2	COX-1	COX-1/ COX-2	Cell type	Reference
Compound A	0.006	1.810	302	CHO	A
Compound B	0.040	15.000	375	Sf9	B
Compound C	0.018	>15.000	>833	CHO	C
Indomethacin	0.026	0.018	0.7	CHO	A

A)= Riendeau *et al.*, Br. J. Pharmacol. 121: 105, 1997

B)= Penning *et al.*, J. Med. Chem. 40: 1347, 1997

C)= Elrich *et al.*, Clin. Pharm. Ther. 65: 336, 1999.

Example 3

Effects of Test Compounds on Volume-Induced Rhythmic Bladder Voiding Contractions in Anaesthetized Rats:

Female Sprague Dawley rats weighing 225-275 g (Crl: CD® BR, Charles River Italia) were used. The animals were housed with free access to food and water and maintained on a 12 h alternating light-dark cycle at 22-24°C for at least one week, except during the experiment. The activity on the rhythmic bladder voiding contractions was evaluated according to the method of Dray (J. Pharmacol. Methods, 13:157, 1985), with some modifications as in Guarneri (Pharmacol. Res., 27: 173, 1993). Briefly, rats were anesthetized by subcutaneous injection of 1.25 g/kg (5 ml/kg) urethane, after which the urinary bladder was catheterized via the urethra using PE 50 polyethylene tubing filled with physiological saline. The catheter was then tied in place with a ligature around the external urethral orifice and connected to a conventional pressure transducer (Statham P23 ID/P23 XL). The

intravesical pressure was displayed continuously on a chart recorder (Battaglia Rangoni KV 135 with DC1/TI amplifier). The bladder was then filled via the recording catheter with incremental volumes of warm (37°C) saline until reflex bladder voiding contractions occurred (usually upon addition of 0.8-1.5 ml saline). After 15 min the test compounds were administered by intravenous (i.v.) route. For i.v. administration of bioactive compounds, a PE 50 polyethylene tubing filled with physiological saline was inserted into the jugular vein.

Bioactivity was assessed in individual animals (using 6-10 rats per dose) by measuring the duration of bladder quiescence (i.e., the duration of time during which no contractions occurred) over a 60 min period. To compare the potency of the tested compounds in inhibiting the bladder voiding contractions, effective doses that prevented bladder contraction for 10 minutes (ED_{10min}) were evaluated by linear regression analysis.

The distension of the urinary bladder in urethane-anaesthetized rats produced a series of rhythmic bladder voiding contractions whose characteristics have been described (Guarneri *et al.*, Pharmacol. Res., 27:173, 1993). Compounds A-C inhibited the voiding contractions, inducing a dose-dependent block of the bladder contractions lasting, at the highest dose tested, up to 34 min. The potency of some selective COX-2 inhibitors in inhibiting bladder voiding contractions (expressed as the dose calculated to induce 10 min of bladder quiescence, *i.e.*, ED_{10min}) was similar (*e.g.*, Compound B) or slightly lower (*e.g.* Compound C) to that of morphine, a drug acting mainly at the level of the central nervous system.

Indomethacin proved less potent than the selective COX-2 inhibitors.

The results are shown in Table 2 below.

Table 2

Effects of COX inhibitors on rhythmic bladder voiding contractions after intravenous administration.

Data represent the mean values ± S.E. of the duration of bladder quiescence (absence of contractions in min). The ED_{10min} values represent the dose inducing a 10 min of absence of bladder contractions, evaluated by linear regression analysis.

	Dose (mg/kg i.v.)	**BLADDER QUIESCENCE** (min ± S.E.)
Compound A	0.1	5.12 ± 1.59
	0.3	11.50 ± 3.99
	1.0	22.52 ± 5.55
	3.0	34.24 ± 13.03
ED_{10min} = 0.208 mg/kg		
Compound B	0.01	4.05 ± 0.68
	0.10	10.53 ±2.04
	1.0	28.35 ± 5.19
ED_{10min} = 0.044 mg/kg		
Compound C	0.03	3.75 ± 0.96
	0.10	4.85 ± 1.80
	0.30	16.44 ± 6.13
ED_{10min} = 0.135 mg/kg		
Indomethacin	0.3	4.39 ± 1.08
	1.0	7.91 ± 1.79
	3.0	35.00 ± 5.78
ED_{10min} = 0.652 mg/kg		
Morphine	0.01	2.76 ± 0.93
	0.03	6.89 ± 2.11
	0.10	13.83 ± 2.40
	0.30	18.17 ± 3.60
ED_{10min} = 0.050 mg/kg		

The results presented in Table 1 and Table 2 demonstrate that Compounds A to C were ineffective inhibitors of the COX-1 isozyme but were very potent inhibitors of both the COX-2 isozyme and bladder voiding contractions. Potency in inhibiting voiding contractions, therefore, was highly correlated with potency in inhibiting the COX-2 isozyme, but was not correlated with potency of COX-1 inhibition.

Summarizing, compounds A to C were found to be selective inhibitors of COX-2 isozyme, exhibiting an inhibitory potency nearly equal to indomethacin (a non selective COX inhibitor) on this isoform of cyclooxygenase, and ineffective inhibitors of the COX-1 isozyme. Compounds A to C were further found to be more potent than indomethacin in inhibiting bladder voiding contraction in anaesthetized rats. The frequency of bladder voiding contractions in anaesthetized rats is related to the sensory afferent arm of reflex micturition and to the integrity of the micturition center (Maggi *et al.*, Brain Res., 415: 1, 1987; Maggi *et al.*, J. Urol., 136:696, 1986). Taking into account the lack of effects of these compounds on the COX-1 isozyme and the presence of constitutive expression of COX-2 in the central nervous system, it is possible that these compounds act at the level of the micturition center to prevent bladder contraction.

Example 4

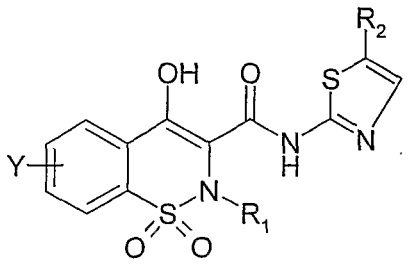
Effects of Test Compounds in Patients Suffering from Lower Urinary Tract Symptoms:
The efficacy of compounds A, B and C in treating lower urinary tract symptoms is tested in patients. Compounds A, B and C are administered orally 1-2 times per day in doses of 5, 12.5, 25 and 100 mg, for a period of 40 days. Daily dosages totaled 5, 10, 12.5, 25, 50, 100 and 200 mg.
The therapeutic effect of compounds A, B and C is measured by patient questionnaires, which are used to determine, e.g., urinary frequency, nocturia, urgency, difficulty with voiding, and pain or discomfort in lower abdominal or genital areas. Effectiveness of compounds A, B and C is measured in amelioration of any symptom associated with LUTS, as compared to a control patient group that is administered placebo, according to the same regimen.

Example 5

Effects of Test Compounds in Patients Suffering from Lower Urinary Tract Symptoms:
The efficacy of compounds D and E in treating lower urinary tract symptoms is tested in patients using the protocol described in Example 4.

CLAIMS

1. Use for the preparation of a medicament for the treatment of neuromuscular dysfunction of the lower urinary tract of a COX-2 selective compound of the general formula I



(I)

or a pharmaceutically acceptable salt thereof, wherein

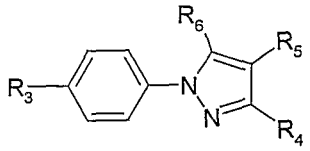
R_1 represents a hydrogen atom or a methyl or ethyl group,

R_2 represents a methyl, ethyl or n-propyl group, and

Y represents a hydrogen, fluorine or chlorine atom or a methyl or methoxy group.

2. Use according to claim 1 of 1,1-dioxo-4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2*H*-1,2-benzothiazine-3-carboxamide (Compound A).

3. Use for the preparation of a medicament for the treatment of neuromuscular dysfunction of the lower urinary tract of a COX-2 selective compound of the general formula II



(II)

or pharmaceutically acceptable salt thereof, wherein

R_3 represents a $NHS(O)_2CH_3$, $S(O)_2CH_3$, $S(O)_2NH_2$, $S(O)(NH)NH_2$ or $S(O)_2NHC(O)(CH_2)_{1\text{-}3}CH_3$ group;

R_4 represents a halogen atom or a haloalkyl, cyano, nitro, aminocarbonyl, N-alkylaminocarbonyl, N,N-dialkylaminocarbonyl, cycloalkylaminocarbonyl, haloaralkyl, aminocarbonylhaloalkyl, alkylsulfonyl or N-alkylaminosulfonyl group;
R_5 represents a hydrogen or halogen atom or an alkyl, thio, alkylthio, haloalkyl, cyano, hydroxyalkyl, alkylsulfonyl or cycloalkyl group; and
R_6 represents an aryl, cycloalkyl, cycloalkenyl or heterocyclic group and is optionally substituted with one or more radicals selected from halogen atoms and alkylthio, alkyl, alkenyl, alkylsulfonyl, cyano, aminocarbonyl, haloalkyl, hydroxyl, alkoxy, hydroxyalkyl, haloalkoxy, sulfamyl, amino, heterocyclic, cycloalkylalkyl and nitro groups; or
R_5 and R_6 together with the pyrazole ring atoms to which they are attached form a 6- or 7-membered ring in which the ring atom next to that in the 4-position in the pyrazole ring is a sulfur atom, the 6- or 7-membered ring optionally being fused with a benzene ring which itself may be substituted with one or more halogen atoms and/or C_{1-4} alkyl groups.

4. Use according to claim 3 of a compound II in which R_3 represents a $S(O)_2NH_2$ group.

5. Use according to claim 3 or claim 4 of a compound II in which R_4 represents a fluorine atom or a trifluoromethyl group.

6. Use according to any of claims 3 to 5 of a compound II in which R_5 represents a hydrogen or halogen atom.

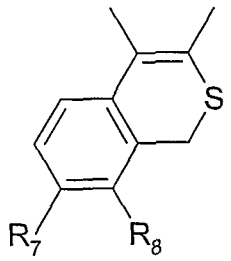
7. Use according to any of claims 3 to 6 of a compound II in which R_6 represents a phenyl group, optionally substituted with one or more halogen atoms and/or C_{1-4} alkyl, C_{2-4} alkenyl, C_{1-4} haloalkyl, hydroxyl, C_{1-4} alkoxy, hydroxyalkyl, haloalkoxy or amino groups.

8. Use according to any of claims 3 to 6 of a compound II in which R_6 represents a p-tolyl group.

9. Use according to claim 3 or claim 4 of a compound II in which R_4 represents a trifluoromethyl group, R_5 represents a hydrogen atom and R_6 represents a para-substituted phenyl group.

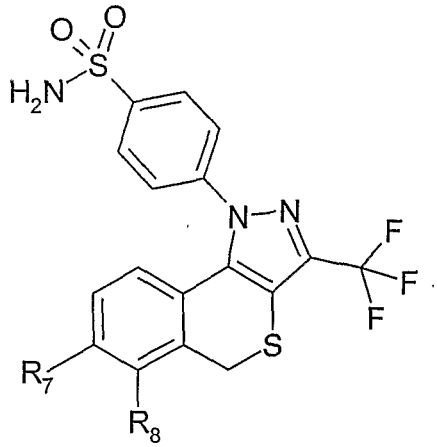
10. Use according to claim 3 of 1-(4-sulfamoylphenyl)-3-trifluoromethyl-5-(p-tolyl)-pyrazole (Compound B).

11. Use according to any of claims 3 to 5 of a compound II in which R_5 and R_6 together with the carbon atoms of the pyrazole ring to which they are attached form a six membered sulfur-containing heterocyclic ring which is further fused to a substituted benzene ring as represented by the structure



wherein R_7 represents a C_{1-4} lower alkoxy group and R_8 represents a halogen atom.

12. Use according to claim 11 of a compound II having the formula

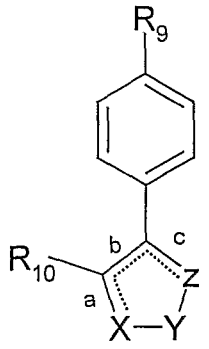


wherein R_7 represents a C_{1-4} lower alkoxy group and R_8 represents a halogen atom.

13. Use according to claim 12 of a compound in which R_7 represents a methoxy group.

14. Use according to claim 12 or claim 13 of a compound in which R_8 represents a fluorine atom.

15. Use for the preparation of a medicament for the treatment of neuromuscular dysfunction of the lower urinary tract of a COX-2 selective compound of the general formula III,



(III)

or a pharmaceutically acceptable salt thereof, wherein

when side b is a double bond and sides a and c are single bonds X-Y-Z represents one of the groups

$-CH_2CH_2CH_2-$,

$-C(O)CH_2CH_2-$,

$-CH_2CH_2C(O)-$,

$-CR_{11}(R'_{11})-O-C(O)-$,

$-C(O)-O-CR_{11}(R'_{11})-$,

$-CR_{11}(R'_{11})-NR_{12}-C(O)-$,

$-N=CR_{13}-O-$,

$-O-CR_{13}=N-$ and

$-C(O)-NR_{12}-CR_{11}(R'_{11})-$

or when sides a and c are double bonds and side b is a single bond X-Y-

Z represents one of the groups

$=CH-O-CH=$,

$=CH-CR_{11}(R'_{11})-CH=$,

=CH-NR_{12}-CH=,

=N-O-CR_{11}=,

=CH-O-N= and

=N-O-N= ;

R_9 represents a $NHS(O)_2CH_3$, $S(O)_2CH_3$, $S(O)_2NH_2$, $S(O)(NH)NH_2$ or $S(O)_2NHC(O)(CH_2)_{1-3}CH_3$ group;

R_{10} represents a C_{1-6} alkyl or C_{3-7} cycloalkyl group or an unsubstituted or mono-, di- or tri-substituted phenyl or naphthyl group, each substituent for the phenyl or naphthyl group being a halogen atom and/or a C_{1-6} alkyl, C_{1-6} alkoxy, cyano or trifluoromethyl group;

each of R_{11} and R'_{11} independently represents a hydrogen atom or a C_{1-6} alkyl group;

R_{12} represents a hydrogen atom or a trifluoromethyl, C_{1-6} alkyl, hydroxy or C_{1-6} alkoxy group; and

R_{13} represents a hydrogen atom or a trifluoromethyl or C_{1-6} alkyl group.

16. Use according to claim 15 of a compound III in which R_9 represents a $S(O)_2CH_3$, $S(O)_2NH_2$ or $S(O)_2NHC(O)CH_2CH_3$ group.

17. Use according claim 15 or claim 16 of a compound III in which R_{10} represents a methyl, ethyl or optionally mono- or di-substituted phenyl group, each substituent for the phenyl group being a halogen atom and/or a methyl, ethyl, methoxy, ethoxy or trifluoromethyl group.

18. Use according to claim 15 or claim 16 of a compound III in which R_{10} represents a phenyl group.

19. Use according to any of claims 15 to 18 of a compound III in which X-Y-Z represents the group $-C(O)-O-CR_{11}(R'_{11})-$.

20. Use according to claim 19 of a compound III in which R_{11} and R'_{11} both represent hydrogen atoms.

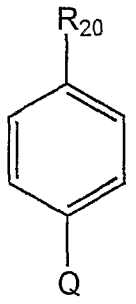
21. Use according to any of claims 15 to 18 of a compound III in which X-Y-Z represents a group

of the formula =N-O-CR_{11}=.

22. Use according to claim 21 of a compound III in which R_{11} represents a methyl group.

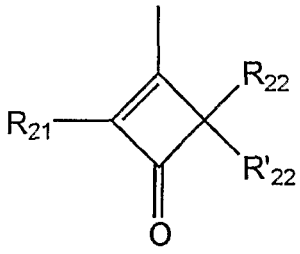
23. Use according to claim 15 of any one of the following compounds:
4-(4-methylsulfonylphenyl)-3-phenyl-2,5-dihydrofuran-2-one (Compound C),
4-(4-sulfamoylphenyl)-5-methyl-3-phenyl-isoxazole (Compound D) and
4-[4-(N-propionylsulfamoyl)-phenyl]-5-methyl-3-phenyl-isoxazole (Compound E).

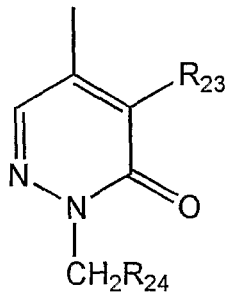
24. Use for the preparation of a medicament for the treatment of neuromuscular dysfunction of the lower urinary tract of a COX-2 selective compound of the general formula V.

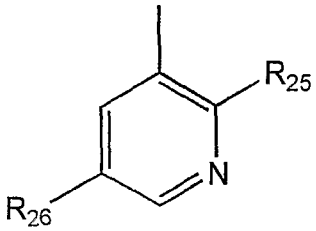


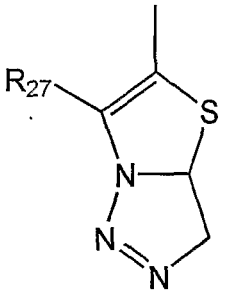
V

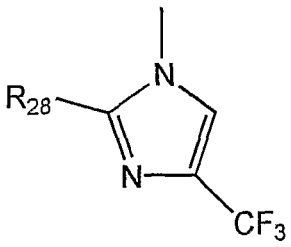
or a pharmaceutically acceptable salt thereof, wherein
R_{20} represents a $NHS(O)_2CH_3$, $S(O)_2CH_3$, $S(O)_2NH_2$, $S(O)(NH)NH_2$ or $S(O)_2NHC(O)(CH_2)_{1-3}CH_3$ group; and
Q represents one of the groups of the formulae Q(i) to Q(v) below

(i) 

(ii) 

(iii) 

(iv) 

(v) 

wherein R_{21} represents a C_{1-6} alkyl or C_{3-7} cycloalkyl group or an unsubstituted or mono-, di- or tri-

substituted phenyl or naphthyl group, each substituent for the phenyl or naphthyl group being a halogen atom and/or a C_{1-6} alkyl, C_{1-6} alkoxy, cyano or trifluoromethyl group;
each of R_{22} and R'_{22} independently represents a hydrogen atom or a C_{1-6} alkyl group;
R_{23} represents a hydrogen atom or a trifluoromethyl, C_{1-6} alkyl, hydroxy, C_{1-6} branched or unbranched alkoxy group;
R_{24} represents a C_{1-6} alkyl or C_{3-7} cycloalkyl group or an unsubstituted or mono-, di- or tri-substituted phenyl group, each substituent for the phenyl group being a halogen atom and/or a C_{1-6} alkyl, C_{1-6} alkoxy, cyano or trifluoromethyl group;
R_{25} represents a C_{1-6} alkyl or C_{3-7} cycloalkyl group or an unsubstituted or mono-, di- or tri-substituted aryl or heteroaryl group, each substituent for the aryl or heteroaryl group being a halogen atom and/or a C_{1-6} alkyl, C_{1-6} alkoxy, cyano or trifluoromethyl group;
R_{26} represents a hydrogen or halogen atom or a C_{1-6} alkyl or C_{1-6} alkoxy group;
R_{27} represents a C_{1-6} alkyl or C_{3-7} cycloalkyl group or an unsubstituted or mono-, di- or tri-substituted phenyl group, each substituent for the phenyl group being a halogen atom and/or a C_{1-6} alkyl, C_{1-6} alkoxy, cyano or trifluoromethyl group; and
R_{28} represents an unsubstituted or mono-, di-, or tri-substituted heteroaryl group.

25. Use according to claim 24 of a compound V in which R_{20} represents a $S(O)_2CH_3$ or $S(O)_2NH_2$ group.

26. Use according to claim 24 or claim 25 of a compound V in which Q represents a Q(i) group wherein R_{21} represents an unsubstituted or mono- or di-substituted phenyl group, each substituent for the phenyl group being a halogen atom and/or a methyl, ethyl, methoxy, ethoxy or trifluoromethyl group.

27. Use according to any of claims 24 to 26 of a compound V in which Q represents a Q(i) group wherein R_{22} and R'_{22} both represent methyl groups.

28. Use according to claim 24 or claim 25 of a compound V in which Q represents a Q(ii) group wherein R_{23} represents a methyl, ethyl, methoxy, ethoxy, n-propoxy or 2-propoxy group.

29. Use according to any of claims 24, 25 or 28 of a compound V in which Q represents a Q(ii) group wherein R_{24} represents an unsubstituted or mono- or di-substituted phenyl group, each substituent for the phenyl group being a halogen atom and/or a methyl, ethyl, methoxy, ethoxy or trifluoromethyl group.

30. Use according to any of claims 24, 25 or 28 of a compound V in which Q represents a Q(ii) group wherein R_{24} represents an unsubstituted or mono- or di-substituted phenyl group, each substituent for the phenyl group being a halogen atom.

31. Use according to claim 24 or claim 25 of a compound V in which Q represents a Q(ii) group wherein R_{23} represents a 2-propoxy group and R_{24} represents a phenyl group.

32. Use according to claim 24 or claim 25 of a compound V in which Q represents a Q(iii) group wherein R_{25} represents a heteroaryl group, optionally substituted with halogen, C_{1-4} alkyl or C_{1-4} alkoxy groups.

33. Use according to claim 24 or claim 25 of a compound V in which Q represents a Q(iii) group wherein R_{25} represents an unsubstituted or mono- or di-substituted pyridinyl group, each substituent for the pyridinyl group being a halogen atom and/or a C_{1-4} alkyl or C_{1-4} alkoxy group.

34. Use according to claim 24 or claim 25 of a compound V in which Q represents a Q(iii) group wherein R_{25} represents a mono- or di-substituted pyridinyl group, each substituent for the pyridinyl group being a halogen atom and/or a methyl, ethyl, methoxy or ethoxy group.

35. Use according to claim 24 or claim 25 of a compound V in which Q represents a Q(iii) group wherein R_{25} represents a 2-methyl-5-pyridinyl group.

36. Use according to any of claims 24, 25 or 32 to 35 of a compound V in which Q represents a Q(iii) group wherein R_{26} represents a chlorine or fluorine atom.

37. Use according to claim 24 or claim 25 of a compound V in which Q represents a Q(iv) group wherein R_{27} represents an unsubstituted or mono-, di- or tri-substituted phenyl group, each substituent for the phenyl group being a halogen atom and/or a C_{1-6} alkyl or C_{1-6} alkoxy group.

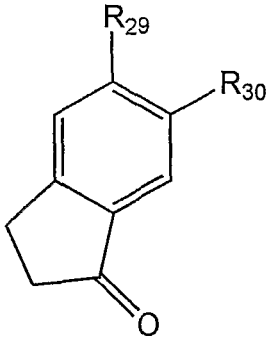
38. Use according to claim 24 or claim 25 of a compound V in which Q represents a Q(iv) group wherein R_{27} represents a phenyl group.

39. Use according to claim 24 or claim 25 of a compound V in which Q represents a Q(v) group wherein R_{28} represents a 3-pyridyl and 4-thiazolyl group, optionally substituted with a C_{1-4} alkyl group.

40. Use according to claim 24 or claim 25 of a compound V in which Q represents a Q(v) group wherein R_{28} represents a 2-methyl-3-pyridyl or 2-methyl-4-thiazolyl group.

41. Use according to claim 24 of any one of the following compounds:
3-(4-methylsulfonylphenyl)-2-(2-methyl-5-pyridyl)-5-chloro-pyridine,
1-(4-methylsulfonylphenyl)-2-(3-pyridyl)-4-trifluoromethyl-imidazole,
1-(4-sulfamoylphenyl)-2-(2-methyl-3-pyridyl)-4-trifluoromethyl-imidazole, and
1-(4-sulfamoylphenyl)-2-(2-methyl-4-thiazolyl)-4-trifluoromethyl-imidazole.

42. Use for the preparation of a medicament for the treatment of neuromuscular dysfunction of the lower urinary tract of a COX-2 selective compound of the general formula VI

VI 

or a pharmaceutically acceptable salt thereof, wherein

R_{29} represents a $NHS(O)_2CH_3$, $S(O)_2CH_3$, $S(O)_2NH_2$, $S(O)(NH)NH_2$ or $S(O)_2NHC(O)(CH_2)_{1-3}CH_3$ group, and

R_{30} represents a C_{1-6} alkyl or C_{3-7} cycloalkyl group or an unsubstituted or mono-, di- or tri-substituted phenyl or phenylthio group, each substituent for the phenyl or phenylthio group being a halogen atom and/or a C_{1-6} alkyl, C_{1-6} alkoxy group, cyano or trifluoromethyl group.

43. Use according to claim 42 of a compound VI in which R_{29} represents a $NHS(O)_2CH_3$, $S(O)_2CH_3$ or $S(O)_2NH_2$ group.

44. Use according to claim 42 or claim 43 of a compound VI in which R_{30} represents a mono-, di- or tri-substituted phenyl or phenylthio group, each substituent for the phenyl or phenylthio group being a halogen atom and/or a C_{1-6} alkyl or C_{1-6} alkoxy group.

45. Use according to claim 42 or claim 43 of a compound VI in which R_{30} represents an unsubstituted or mono-, di- or tri-substituted phenylthio group, each substituent for the phenylthio group being a halogen atom.

46. Use according to claim 42 or claim 43 of a compound VI in which R_{30} represents a difluorophenylthio group.

47. Use according to claim 42 of 6-(2,4-difluorophenylthio)-5-methylsulfonylamino-1-oxo-2,3-dihydro-indene.

48. Use for the preparation of a medicament for the treatment of neuromuscular dysfunction of the lower urinary tract of a compound that significantly inhibits the COX-2 isozyme and which exhibits an inhibitory potency for the COX-2 isozyme that is at least 10-fold greater than the inhibitory potency said compound exhibits for the COX-1 isozyme.

49. Use according to claim 48 of a compound which exhibits an inhibitory potency for the COX-2

isozyme that is at least 100-fold greater than the inhibitory potency said compound exhibits for the COX-1 isozyme.

50. Use according to claim 48 or claim 49 of a compound that has an IC_{50} of from 0.1 to 100 nM.

51. Use according to any of claims 48 to 50 for the preparation of a medicament in a form suitable for oral, transdermal, enteral, intravenous, intramuscular, subcutaneous, transmucosal or inhalatory administration.

52. Use according to any of claims 48 to 51 for the preparation of a medicament further comprising a pharmaceutically acceptable diluent or carrier.

53. Use according to any of claims 48 to 52 for the preparation of a medicament in a form suitable for administration at a dose of from 0.05 to 50 mg/kg/day.