JPP Journal of Pharmacy and Pharmacology
ROYAL PHARMACEUTICAL SOCIETY

JPP Journal of Pharmacy and Pharmacology

JPP 2011, 63: 95–105

Received January 20, 2010
Accepted July 6, 2010
DOI
10.1111/j.2042-7158.2010.01169.x
ISSN 0022-3573

Research Paper

Relative similarity within purine nucleotide and ligand structures operating on nitric oxide synthetase, guanylyl cyclase and potassium (K_{ATP}, BK_{Ca}) channels

W. Robert Williams

Faculty of Health, Sport & Science, University of Glamorgan, Cardiff, UK

Abstract

Objectives Purine nucleotides play a central role in signal transduction events initiated at the cell membrane. The NO–cGMP–cGK pathway, in particular, mediates events involving NOS and some classes of K^+ ion channel. The aim of this study is to investigate relative molecular similarity within the ligands binding to NOS, K_{ATP}, BK_{Ca} channels and regulatory nucleotides.

Methods Minimum energy conformers of the ligand structures were superimposed and fitted to L-arginine and the nucleotides of adenine and guanine using a computational program.

Key findings Distinctive patterns were evident in the fitting of NOS isoform antagonists to L-arginine. K_{ATP} channel openers and antagonists superimposed on the glycosidic linkage and imidazole ring of the purine nucleotides, and guanidinium and ribose groups of GTP in the case of glibenclamide. The fits of BK_{Ca} channel openers and antagonists to cGMP were characterized by the linear dimensions of their structures; distances between terminal oxy groups in respect of dexamethasone and aldosterone.

Conclusions The findings provide structural evidence for the functional interaction between K^+ channel openers/antagonists and the regulatory nucleotides. Use of the purine nucleotide template systematizes the considerable heterogeneity evident within the structures of ligands operating on K^+ ion channels.

Keywords guanylyl cyclase; nitric oxide synthetase; potassium channels; purine nucleotides; structure–activity relationships

Introduction

Nitric oxide generation by nitric oxide synthetase (NOS) functions as a major regulator of the signal transduction processes transmitted from cell receptors and ion channels. Many of the effects of NO relate to the guanine nucleotide–cyclic nucleotide transition cycle initiated by soluble guanylyl cyclase. The intracellular targets of cGMP include cGMP-dependent protein kinases (cGK), phosphodiesterases and cyclic nucleotide-gated ion channels. The substrates phosphorylated by cGKs are components of ion channels, G-proteins and cytoskeleton-associated proteins found in cells of the nervous system, cardiovascular system and other organs.[1,2]

The isoforms of NOS have different cell and tissue distribution (endothelial (eNOS), neuronal (nNOS), inducible (iNOS)) but share similar enzymic and pharmacologic properties with binding sites for heme, substrate, co-factors and calmodulin.[3,4] Substrate binding of L-arginine occurs through hydrogen-bonding of guanidinium and amino acid moieties to the protein residues. Effective NOS inhibitors require a suitable binding scaffold for the guanidinium pocket and extending functional groups to exploit the conformational differences between the isoforms.[3]

Linkage of the K_{ATP} channel and NOS pathway is evident in the activation of K_{ATP} channels via NO–cGMP–cGK transduction mechanisms.[5,6] Acetylcholine and bradykinin promote the pre-conditioning of cardiac tissue by opening mitoK_{ATP} channels, which involves the receptor-mediated production of NO, generation of cGMP, activation of protein kinases and release of reactive oxygen species (ROS).[7] ROS generation by pharmacologic openers of the K_{ATP} channel (nicorandil, pinacidil, 8-Br-cGMP, S-nitroso-N-acetylpenicillamine) is inhibited by a heterogeneous group of antagonists that include inhibitors of the mitoK_{ATP} channel (5-hydroxydecanoate) and soluble guanylyl cyclase

Correspondence: W. Robert Williams, Faculty of Health, Sport & Science, University of Glamorgan, Cardiff CF371DL, UK.
E-mail: wrwillia@glam.ac.uk

(ODQ),[8] an NOS inhibitor (L-NAME)[9] and K_{ATP} channel antagonist (glibenclamide).[10] As guanine and adenine nucleotides are established endogenous regulators of the mitoK_{ATP} channel, the pharmacologic properties of modulators of NOS and the K_{ATP} channel may be evident within the purine nucleotide structures.[11]

Linkage of the K_{ATP} channel with the NOS/cGMP pathway is not exclusive, as there is a guanine nucleotide promoted link between NOS and the BK_{Ca} channel.[12] Both NOS and BK_{Ca} channel proteins are modulated by steroids. Aldosterone increases BK_{Ca} channel expression in colonic tissue and produces an acute NO-mediated dilation of rat arterioles, susceptible to antagonism by spironolactone or L-NAME.[13,14] Dexamethasone modulates BK_{Ca} channel gating in defined cell lines by a non-genomic mechanism.[15,16] 17β-Estradiol and testosterone open BK_{Ca} channels in human coronary and corporal smooth muscle cells, respectively.[17,18]

In endothelial cells, acetylcholine, histamine, adenosine and clonidine activate NOS via specific cell membrane receptors and there is some evidence for receptor activation by L-arginine.[19,20] The signal-transducing pharmacophores within these small molecular weight compounds together with the reported interaction between ligands of NOS, guanylyl cyclase and K^+ channels, suggests some commonality in molecular structure. Further insight into relative molecular similarity within endogenous compounds and drug structures is required to identify the biochemical deficits in tissue that require intervention by pharmaceutical agents following pathologic change or ageing.

The aim of this study is to provide new evidence, using a molecular modelling approach, of molecular similarity within the wide range of heterogeneous ligands targeting NOS and K^+ channels. The templates selected for the comparative study are the purine nucleotides that provide transduction links from cell receptor proteins.

Materials and Methods

Chemical compounds

The molecular structures of the investigated compounds with established activity at NOS and potassium channel sites were from the following sources: NOS inhibitors (emdbiosciences.com), K_{ATP} channel ligands,[21] Pubchem (http://pubchem.ncbi.nlm.nih.gov). The compounds were selected on the basis of their target affinity and selectivity, and representation of different chemical classes.

NOS inhibitors categorised by isoform specificity

Non-selective: S-ethylisothiourea, 1-[2-(trifluoromethyl) phenyl]1H-imidazole (TRIM), 7-nitroindazole; *e-NOS*: N^G-nitroarginine, N^5-(1-iminoethyl)ornithine; *n-NOS*: N^G-propyl-arginine, thiocitrulline, (4S)-*N*(4-amino-5(aminoethyl) aminopentyl-*N*-nitroguanidine (NOS-neuronal inhibitor 1);[22] *i-NOS*: aminoguanidine, *N*-(3-(aminomethyl)benzyl) acetamidine (1400W), 7-chloro-3-imino-5-methyl-2-azabicyclo-[4.1.0] heptane (ONO1714),[23] 2-(2-(4-methoxypyridin-2-yl)ethyl)-3H-imidazo(4,5-b)pyridine (BYK 191023),[24] S,S'-(1,3-phenylenebis (1,2 ethanediyl)) bisisothiourea (1,3-PBITU).[25]

B_{KCa} channel ligands

Propofol,[26] *N*-(2-hydroxy-5-phenyl)-(2-methox-5-chloro)-benzamide (benzanilide16b),[27] benzofuroindole22,[28] magnol, kaempferol, paxilline,[29] clotrimazole,[30] OR1896.[31]

K_{ATP} channel ligands

(R)-Pinacidil, gliclazide, glibenclamide (S)-5-hydroxydecanoate, diazoxide (3S,4R)-levcromakalim, isoflurane, *N*-cyano-*N*'-(1,1-dimethylpropyl)-*N*''-(pyridinyl)guanidine (P1075),[32] iptakalim,[33] BMS-191095,[34] (S)-PNU-99963.[35]

Miscellaneous stuctures

Arginine, aldosterone, choline, ATP, cGMP, GTP, dexamethasone, clonidine, ethyl 3,4-dihydroxybenzoate, muscarine, S-nicotine, rauwolscine, spironolactone, 17-β-estradiol, testosterone.

Modelling

Charge calculations, conformational analysis, and the building, superimposition and fitting of molecular structures were undertaken using the Nemesis program (Oxford Molecular version 2.1). Low energy conformers of ATP and GTP were generated from structures in the Nemesis library file, and the remaining structures were built from contents of the program fragment file. Minimum energy conformers were obtained by conformational analysis and minimisation within the Nemesis program. Molecular structures were fitted to the purine nucleotide and arginine templates on the basis of a 3-point fit of atoms of similar type, inter-atomic distance and partial charge. Amino acid and dipeptide structures were in their charged forms. The sequence of fitting was that given in the tables (order from left to right) for each structure. Quality of fit was expressed in terms of inter-atomic distance and root mean square (RMS), which were values computed automatically by the Nemesis program.

Torsion angles of minimum energy conformers

NOS ligands

Arginine O2C1C2C3 −84, O2C1C2N1 153, C1C2C3C4 −46, C2C3C4C5 −63, C3C4C5N2 −51, C4C5N2C6 130, C5N2C6N3 144. *Imino-ornithine* O2C1C2C3 −84, O2C1C2N1 153, C1C2C3C4 −44, C2C3C4C5 −65, C3C4C5N2 −53, C4C5N2C6 124 C5N2C6C3 151. *Nitro-arginine* O2C1C2C3 −81, O2C1C2N1 155, C1C2C3C4 −41, C2C3C4C5 −71, C3C4C5N2 −54, C4C5N2C6 103, C5N2C6N3 −167, N2C6N3N7 136, C6N3N7O3 −151. *S-Ethylisothiourea* C2C3S1C6 −180; C3S1C6N3 179. *Thio-citrulline* O2C1C2C3 −44, O2C1C2N1 −167, C1C2C3C4 −54, C2C3C4C5 −59, C3C4C5N3 −39, C4C5N3C6 164; C5N3C6N2 137. *NOS-neuronal inhibitor 1* O3N5N3C6 95, N5N3C6N2 −131, N3C6N2C5 151, C6N2C5C4 −109, N2C5C4C3 46, C5C4C3C2 81, C4C3C2C1 173, C3C2C1N1 169, C2C1N1C7 −142, C1N1C7C8 96, N1C7C8N8 −14. *Propyl-arginine* O2C1C2C3 −85, O2C1C2N1 153, C1C2C3C4 −45, C2C3C4C5 −64, C3C4C5N2 −52, C4C5N2C6 130, C5N2C6N3 144, N2C6N3C7 −173, C6N3C7C8 76, N3C7C8C9 179. *1400W* C7C6N2C5 5, C6N2C5C1 72, N2C5C1C2 −156. *TRIM* C6N3C5C1 −73. *1,3-PBITU* N3C6S2C7 −165, C6S2C7C8 61, S2C7C8C1 46,

C7C8C1C2 –98, C2C3C4C5 60, C3C4C5S1 –180, C4C5S1C9 167, C5S1C9N5 169. *BYK 191023* N3C6C7C8 75, C6C7C8C1 –56, C7C8C1N5 –37. *Choline* C3N6C2C5 –172, N6C2C5O1 –53. *Muscarine* C2C7C8N6 –164. *Nicotine* C7C6C2N6 113. *Clonidine* C7C8N5C6 –149. *Rauwolscine* C10O3C9C7 –176, O3C9C7C8 –72. *Dihydroxy ethylbenzoate* O5C6C1C2 180.

K_{ATP} ligands

Iptakalim C3C2C1C4 –177, C3C2C1N9 –53, C2C1N9C8 –65, C1N9C8C9 –100. *Levcromakalim* C9N9C1C7 60. *BMS-191095* C12C11N9C1 –102, C12C11N9C3 –105, C12C11N9N3 122, C11N9C7C8 –66, N9C3C4N3 141. *P1075* C2C1N9C8 –35, C1N9C8N7 –173, N9C8N7C9 13, C8N7C9N6 –4, C1N9C8N5 7, N9C8N5C11 180, C8N5C11C12 –173. *PNU-99963* C2C1N7C8 –143, C1N7C8N5 –122, N7C8N5C7 1, C8N5C7N6 –14, C1N7C8N9 60, N7C8N9C9 20, C8N9C9 C10 –98, N9C9C10C11 –22. *Pinacidil* C6C5N10C8 159, C5N10C8N8 175, N10C8N8C7 0, C8N8C7N6 174, C5N10C8N9 –11, N10C8N9 C1 171, C8N9C1C9 108, N9C1C9C10 59. *Isoflurane* F1C4O9C1 –176, C4O9C1C5 175, O9C1C5F4 –60. *Glibenclamide* C17C16N7C8 –76, C16N7C8N9 –4, N7C8N9S1 –174, C8N9S1C1 –72, N9S1C1C2 –2, C3C4C5C6 105, C4C5C6N5 –42, C5C6N5C7 –70, C6N5C7C9 153, N5C7C9C10 180. *Gliclazide* C9N6N7C8 105, N6N7C8N9 –0, N7C8N9S1 –177, C8N9S1C1 –53, N9S1C1C2 –85.

5-Hydroxydecanoate C5C6C7C8 –78, C6C7C8C9 70, C7C8C9C1 174, C8C9C1C2 179, C9C1C2C3 179, C1C2C3C4 –180, C2C3C4C5 –180.

B_{KCa} ligands

Benzanilide 16b C6C5C4N9 179, C5C4N9C7 180, C4N9C7C9 2; *magnol* C6C5C4C3 140; *OR1896* C11C10C7N5 8; *clotrimazole* C9N9C4C5 172, N9C4C2C3 –74, N9C4C5C6 –43, N9C4C7C8 15; *kaempferol* C5C411C12 –8.

Miscellaneous structures

GTP O9C1′N9C8 –47. *ATP* O9C1′N9C8 –38. *cGMP* O9C11N9C8 –33.

Results and Discussion

Modulators of NOS

As most of the listed structures provide an almost exact fit to the L-arginine conformer, only those with fits of lower tolerance (interatomic distances ≥0.1 Å, RMS values ≥0.0100) are given in Table 1. Ethylisothiourea and aminoguanidine (Figure 1, structures f and m, respectively) represent the minimum structures for antagonism of NOS and direct the main focus of fitting to the guanidinium group of arginine. The guanidinium group is represented by an amidinocarbon in the iminoethylornithine structure. Iminoethylornithine and nitroarginine are competitive inhibitors of arginine, as small changes in the α-amino acid or guanidinium moieties are sufficient to inhibit NO formation.[36] Other structures, including the imidazole components of TRIM, BYK 191023 and clonidine, have a substitute guanidinium group as part of a heterocyclic ring. Berka *et al*.[37] provide evidence for the direct binding of 2-amino imidazole to the guanidine binding sub-domain of L-arginine. The fits of nNOS isoform inhibitors (structures f–k) to the guanidinium group of arginine place their residual structures in the 6 o'clock position with reference to the N3 fitting point. In contrast, the residual moieties of the iNOS isoform inhibitors (structures l–r) lie within the 11 to 3 o'clock segment with reference to fitting point C6, though the imidazopyridine moiety of BYK 191023 creates a southerly extension of the structure. There is no relationship between the quality of fit of the NOS antagonists and their known potencies for inhibition of NOS. Differences in the potency of NOS inhibitors are influenced significantly by restrictions on access to the isoform sites. Isoform active-site cavity volumes in the region of the guanidinium binding pocket account for the selectivity of structures like *N*-propyl-L-arginine for the larger binding pocket of nNOS.[3] Furthermore, NOS inhibitors assume different conformations at different sites. Dipeptide inhibitors adopt curled and extended conformations at nNOS and eNOS sites, respectively.[4]

The compounds in Figure 2 have established effects on NOS activity. Promotion of NO synthesis by small molecular weight agonists of different receptor classes and their antagonism by L-NAME[19] supports enquiry into relative similarity within agonist, arginine and guanine nucleotide structures. The requirement for an external supply of arginine for NO synthesis, despite high intracellular levels (the arginine paradox) is indicative of a hormone-like requirement for the amino acid in the plasma membrane.[38] The fits of choline (a full agonist of NOS), muscarine, nicotine and clonidine are of good quality and contained within the arginine structure (Table 2). In rats, the induction of an antipressor response to L-NAME and spinal analgesia, by nicotinic and muscarinic agonists, respectively, are mediated via the NO/cGMP pathway.[39,40] Clonidine has a

Table 1 Fitting data of NOS ligands and arginine

Structure	Fitting points	Interatomic distance (Å)	RMS value
Ethylisothiourea (e)	C6N3C2	0.17 0.12 0.05	0.0249
Ethylisothiourea (f)	N4C6S3	0.15 0.15 0.30	0.0032
Ethylisothiourea (l)	N4N3C5	0.15 0.21 0.17	0.0707
7-Nitroindazole (d)	N4C6C1	0.10 0.17 0.11	0.0218
7-Nitroindazole (k)	N4C6C3	0.06 0.08 0.05	0.0283
Thiocitrulline	S4C6N3	0.25 0.15 0.11	0.0100
TRIM	N4C6N3	0.08 0.08 0.08	0.0300
BYK 191923	N4C6N3	0.05 0.06 0.06	0.0219

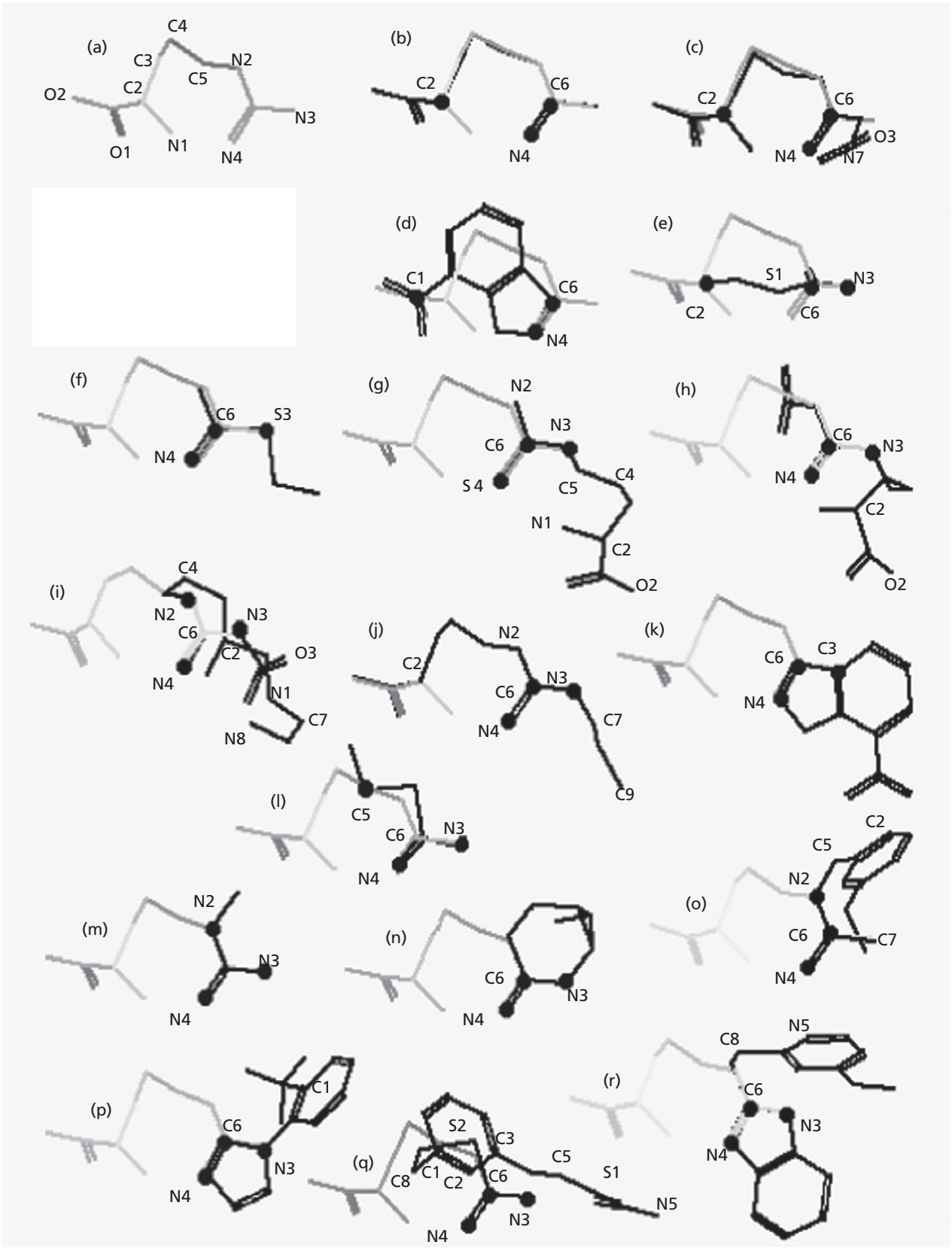

Figure 1 Fitting of NOS antagonists (black) to arginine (grey): arginine (a), iminoethylornithine (b), nitroarginine (c), 7-nitroindazole (d), ethylisothiourea (e and f), thiocitrulline (g), nitroarginine (h), NOS-neuronal inhibitor 1 (i), propyl arginine (j), 7-nitroindazole (k), ethylisothiourea (l), aminoguanidine (m), ONO1714 (n), 1400W (nsp3) (o), TRIM (p), 1,3, PBITU (q), BYK 191023 (r).

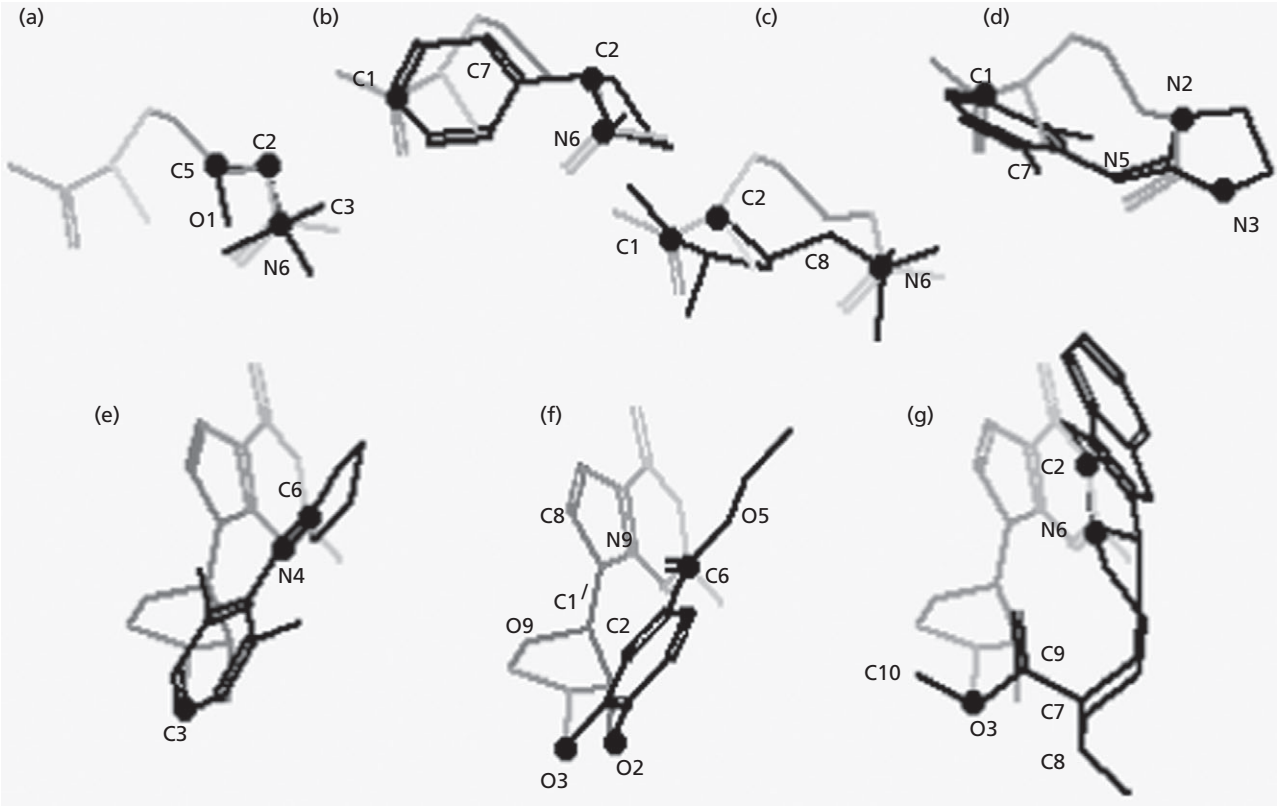

Figure 2 Fitting of receptor agonists/antagonists (black) to arginine (a–d) (grey) or GTP (e–g) (grey): choline (a), nicotine (b), muscarine (c), clonidine (d, e), dihydroxyethylbenzoate (f), rauwolscine (g). The triphosphate moiety of the GTP conformer is deleted to improve on presentation.

Table 2 Fitting data of NOS ligands and arginine or GTP

Structure	Fitting points	Inter-atomic distances (Å)	RMS value
Choline	N6C2C5	0.09 0.06 0.07	0.0068
Nicotine	N6C2C1	0.15 0.16 0.05	0.0249
Muscarine	N6C2C1	0.03 0.03 0.01	0.0004
Clonidine (d)	N3N2C1	0.23 0.07 0.21	0.0296
Clonidine (e)	N4C6C3	0.06 0.12 0.13	0.0111
Dihydroxyethylbenzoate	C6O3O2	0.06 0.08 0.15	0.0017
Rauwolscine	N6C2O3	0.11 0.02 0.09	0.0044

dual action on NO release via muscarine/nicotinic receptors and the α_2-adrenergic receptor/NO-cGMP pathway.[41,42] Joshi *et al*.[20] discuss relative similarity within the structures of arginine, imidazoline and α_2-adrenoceptor ligands (clonidine and rauwolscine) in terms of the guanidinium group and modulation of NO synthesis via ligand binding to cell membrane receptors. In Table 2, clonidine, ethyl dihydroxybenzoate and rauwolscine provide similar high quality fits to the guanidinium group and ribose ring hydroxyls of the GTP conformer. The fit of ethyl dihydroxybenzoate is relevant to the opening of the mitoK_{ATP} channel via activation of the NOS–guanylyl cyclase–PKG cascade.[6] Modulation of NOS and guanylyl cyclase enzyme activity at active centres and allosteric sites may be based on the described molecular similarity relative to their substrate structures.

K_{ATP} channel ligands

The K_{ATP} channel ligands listed in Table 3 demonstrate a high degree of tolerance in their fits to the ATP and GTP nucleotides. Similarity within the carbon skeleton of iptakalim (Figure 3) and structure of pinacidil is evident in competitive binding studies.[43] The geometry of iptakalim and isoflurane relates to the nucleoside glycosidic linkage. This feature is also characteristic of the cyanoguanidine drugs (pinacidil, P1075) and benzopyran derivatives (levcromakalim, BMS-191095). The latter compound, described as a highly selective opener of the mitoK_{ATP} channel, provides a similar fit to diazoxide. Similarity within the compound structures identified in Figure 3 is evident in drug binding studies as competition between the various ligands (P1075,

Table 3 Fitting data for K_{ATP} channel ligands and ATP or GTP

Structure	Fitting points	Inter-atomic distances (Å)	RMS value
Iptakalim	N9C1C2	0.03 0.02 0.03	0.0001
Levcromakalim	N9C1C2	0.01 0.01 0.01	0.0044
P1075	N9C8N7	0.06 0.04 0.07	0.0160
Pinacidil	N9C8C1	0.00 0.00 0.00	0.0007
BMS-191095	C1N9C2	0.04 0.03 0.02	0.0076
PNU99963	N9C8N7	0.08 0.05 0.09	0.0227
Isoflurane	C1O9C4	0.05 0.04 0.05	0.0181
Glibenclamide (k)	N9C8N7	0.08 0.07 0.09	0.0263
Gliclazide (j)	N9C8N7	0.02 0.03 0.03	0.0062
5-Hydroxydecanoate[a]	C8C5C1	0.09 0.14 0.05	0.0177
Glibenclamide (l)[a]	C6N3O3	0.16 0.08 0.08	0.0181
Diazoxide[a]	N9C8C3	0.03 0.07 0.08	0.0066
Gliclazide (m)[a]	C6N3N2	0.02 0.03 0.04	0.0094

[a]Fit of ligand to GTP.

levcromakalim, glibenclamide) of the K_{ATP} channel.[44] The K_{ATP} channel blocker PNU-99963 superimposes more fully on the nucleotide pyrimidine ring in comparison to the fits of the channel openers. Association of K^+ channel blocking activity with the pyrimidine ring of the nucleotide base may also be relevant to the properties and structure of 4-aminopyridine (not shown).

The channel openers provide the same fits to the adenine and guanine nucleotides on account of equivalent fitting points in the ribose and imidazole moieties. This is in contrast to the K_{ATP} channel antagonists, which influence base, sugar and phosphate moieties of the nucleotides. Glibenclamide fits to the guanine nucleotide, in contrast to the more restricted fit of gliclazide to the guanine base. This difference in the fitting of the K_{ATP} antagonists is in keeping with the observation that gliclazide, unlike glibenclamide, does not abolish the cardioprotective effect of diazoxide on the mitoK_{ATP} channel.[45] Glibenclamide blocks both the direct vasodilatory effect of pinacidil and indirect effect of NO on activation of the K_{ATP} channel in the coronary endothelium.[46] The interaction between these diverse compounds is readily understandable if their effects are considered to be mediated via nucleotide regulation of the K_{ATP} channel. Guanine nucleotides competitively reverse regulation of the K_{ATP} channel by adenine nucleotides.[47,48] Nucleotide sensitivity of the K_{ATP} channel is not fixed but modulated by cellular factors[21,49] that may also influence drug action, such as the binding of glibenclamide to the sulphonylurea receptor.[44]

A recent study on the skeletal muscle K_{ATP} channel discusses the ATP-dependent opening and blocking properties of potent 1,4-benzoxazine derivatives in terms of their influence on ATP/ADP binding sites on SUR subunits and demonstrates the overlay of the derivatives on the structure of ATP.[50] This author confirms the relative molecular similarity identified within the ATP and 1,4-benzoxazine structures. Channel opening benzoxazine derivatives use the same fitting points as P1075 (Figure 3); the oxazine moiety superimposes on the ribose ring and the purine ring is affected only by the fitting point atoms of the pyridylamine group (data not shown). The blocking properties of the same benzoxazine derivatives (observed in the absence of ATP) can be accommodated by an alternative fit with the oxazine ring fitting the purine ring and the pyridine ring superimposed on the ribose ring of ATP.

Animal experimentation has established a link between the arginine–NO–cGMP–cGK pathway and role of mitoK_{ATP} channels in providing cardioprotection.[5] Pre-conditioning is controlled pharmacologically by initiators (diazoxide, 8-BrcGMP, ethyl dihydroxybenzoate) and inhibitors (L-NAME, ODQ, glibenclamide, 5-hydroxydecanoate) of pathways that directly or indirectly modulate the GTP/cGMP transition state.[6,10] Three types of allosteric coupling effects may explain the interaction between nucleotide, sulphonylurea and opener binding to the sulphonylurea receptor of the K_{ATP} channel.[51] The current findings demonstrate that although the drug classes are dissimilar, their functional properties may be rationalised through structural similarity to the regulatory purine nucleotides.

BK_{Ca} channel ligands

The opening of BK_{Ca} channels by 17β-estradiol in coronary arteries is mimicked by cGMP, which is the reference structure used in the fitting of the BK_{Ca} channel ligands (Figure 4).[52] Propofol is one of the least complex of the diverse compounds representing the BK_{Ca} channel openers. Magnol, benzanilide16b and OR-1896 may be regarded as linear extensions of the propofol structure with dumbell-like shapes. Benzofuroindole and the steroid structures are comprised of fused rings with linear dimensions that relate to the cyclic nucleotides. For several of the opener compounds, fitting involves the C4, C5 points in the guanine ring and a third point on the ring or an alternative nucleotide fitting point for the elongated structures. The latter fit with their corresponding planes perpendicular to the nucleotide (structures are duplicated and re-orientated in Figure 4 to identify their fitting points). The BK_{Ca} channel openers and antagonists provide fits of high quality (Table 4). As with the K_{ATP} channel openers, discussed above, fits of the BK_{Ca} channel openers show less superimposition on the guanine ring in comparison with the antagonists, clotrimazole, kaempferol and paxilline. Testosterone and 17β-estradiol provide similar fits to the benzofuroindole structure. BK_{Ca} channel activity is stimulated by testosterone and 17β-estradiol in vascular and smooth muscle

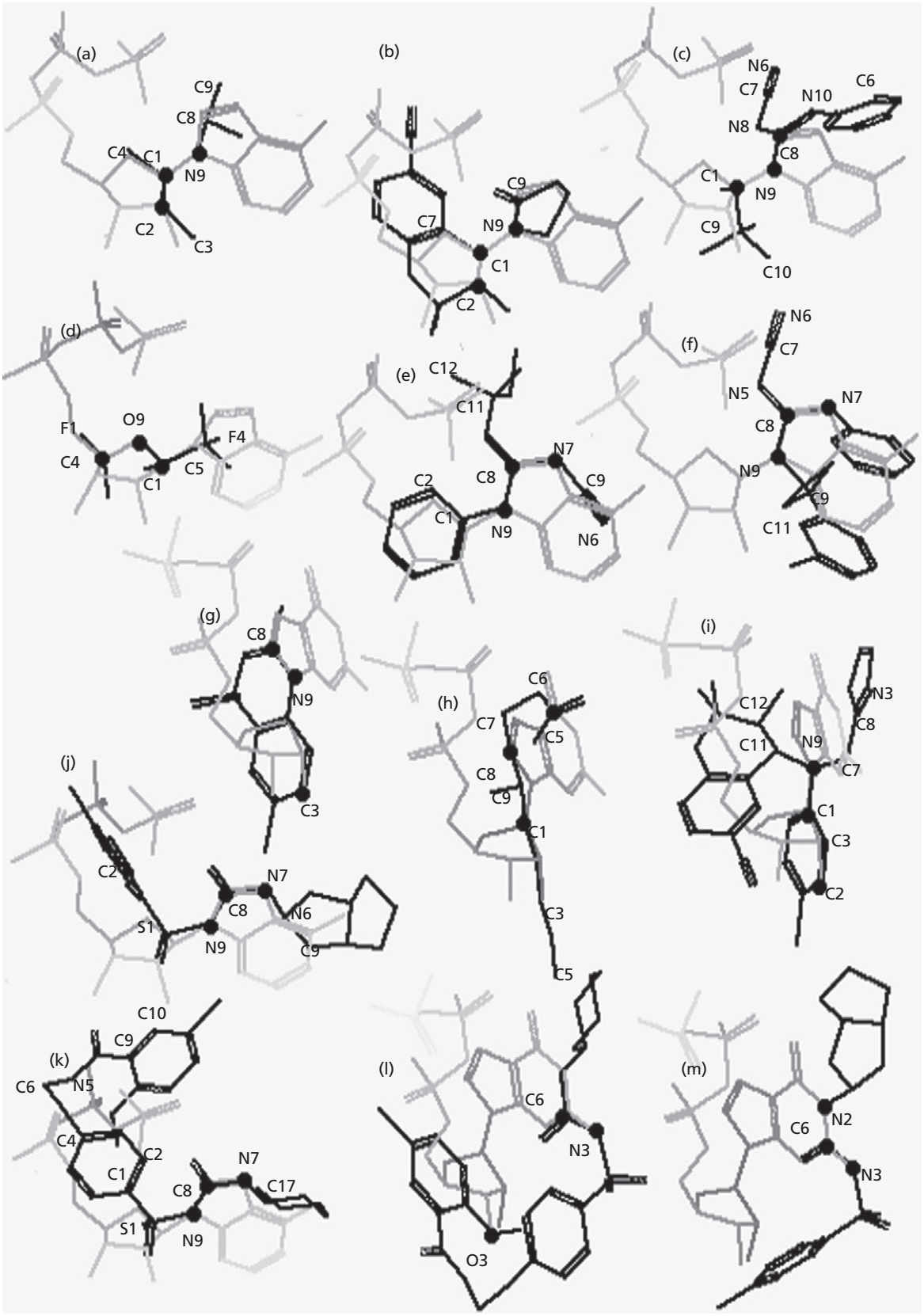

Figure 3 Fitting of K_{ATP} channel ligands (black) to ATP (a–f,j,k) (grey) and GTP (g–i,l,m) (grey): iptakalim (a), levcromakalim (b), pinacidil (c), isoflurane (d), P1075 (e), PNU-99963 (f), diazoxide (g), 5-hydroxydecanoate (h), BMS-191095 (i), gliclazide (j, m), glibenclamide (k, l).

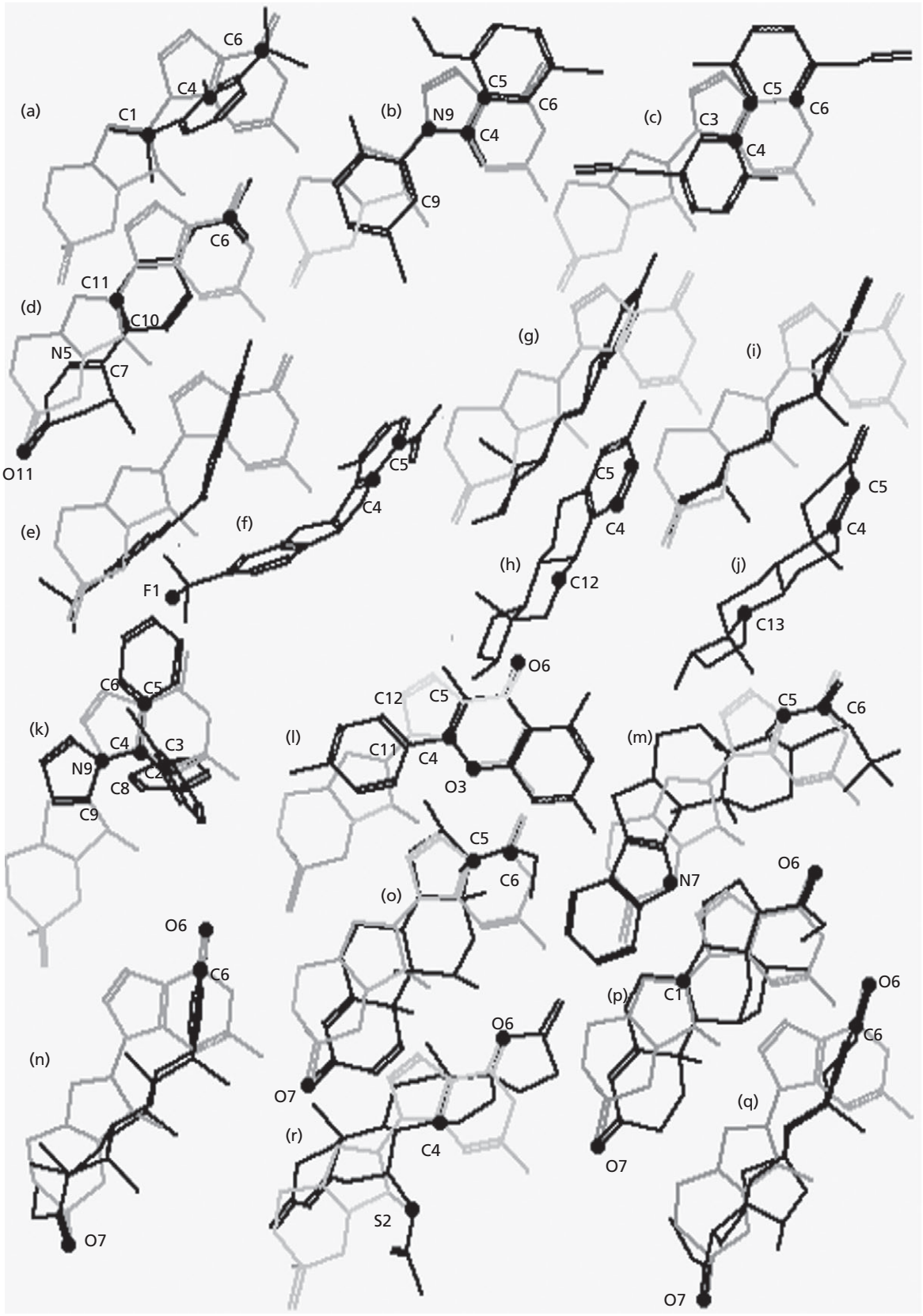

Figure 4 Fitting of B_{KCa} ligands (black) to cGMP (grey): propofol (a), benzanilide 16b (b), magnol (c), OR1896 (d), benzofuroindole (e, f), 17β-estradiol (g, h), testosterone (i, j), clotrimazole (k), kaempferol (l), paxilline (m), dexamethasone (n, o), aldosterone (p, q), spironolactone (r).

Table 4 Fitting data for BK_{Ca} channel ligands and cGMP

Structure	Fitting points	Inter-atomic distances (Å)	RMS value
Propofol	C4C6C1	0.20 0.19 0.16	0.0615
Benzanilide16b	C4C5N9	0.07 0.14 0.12	0.0387
Magnol	C4C5C6	0.06 0.04 0.02	0.0058
OR1896	C11C6O11	0.17 0.08 0.11	0.0405
Benzofuroindole22	C4C5F1	0.04 0.01 0.05	0.0003
17β-Estradiol	C4C5C12	0.05 0.03 0.02	0.0089
Testosterone	C4C5C13	0.07 0.05 0.05	0.0081
Clotrimazole	C4C5N9	0.03 0.17 0.15	0.0173
Kaempferol	C4O6O3	0.01 0.00 0.00	0.0024
Paxilline	C5C6N7	0.03 0.07 0.08	0.0040
Dexamethasone (n)	C6O6O7	0.05 0.07 0.12	0.0007
Dexamethasone (o)	C5C6O7	0.09 0.14 0.07	0.0085
Aldosterone (p)	C1O6O7	0.08 0.15 0.20	0.0235
Aldosterone (q)	C6O6O7	0.18 0.04 0.17	0.0044
Spironolactone	C4O6S2	0.09 0.01 0.09	0.0109

tissue, respectively, albeit by high steroid concentrations.[17,53,54] Testosterone and progesterone influence ion channel currents (I_{KS} and $I_{Ca.L}$) through the non-genomic and cyclic nucleotide dependent modulation of NOS.[55]

Glucocorticoids and mineralocorticoids have obvious but perhaps previously unrecognised structural similarity to cyclic nucleotides, even though their facilitative action in regulating cell function is long recognised.[56] The distances between terminal oxygen atoms in the structures of aldosterone, progesterone and cGMP are, respectively, 11.9 Å, 11.8 Å, 11.7 Å in comparison with 13.0 Å in spironolactone. Dexamethasone gives a high quality fit (structure O), which is replicated by aldosterone (C5C6O7; 0.11 Å, 0.09 Å, 0.17 Å; RMS 0.0105 – not shown) and equivalent to the fit of OR1896. Dexamethasone and aldosterone also provide similar fits (structures n and q) to the gonadosteroids, although obvious differences, in fitting points and positions of residual moieties, are evident. The structures of spironolactone, aldosterone and paxilline superimpose in a similar manner on cGMP (structures m, p, r). The alternative fits of aldosterone equate with the complex properties of this steroid. The acute dilation or contraction of isolated rat arterioles by aldosterone via the NOS pathway is dependent on the integrity of the endothelium and method of administration.[14]

In contrast to the K_{ATP} channel, the BK_{Ca} channel has no recognised nucleotide binding sites supporting a functional role for cGMP that would account for the observed molecular similarity of the nucleotide and BK_{Ca} channel ligands. The established role for cGMP in modulating BK_{Ca} channel function is via the action of cGK and any additional role of cGMP in support of BK_{Ca} channel function may be eclipsed by the effects of cGK. The investigation of potential binding sites for cGMP on the BK_{Ca} channel is facilitated, however, by the binding data of compounds that are the same or of similar structure to those fitted to the cGMP template. In this context, biarylurea derivatives and maxikdiol are known as α-subunit-selective BK_{Ca} openers.[57] The α-subunit of the BK_{Ca} channel is the substrate site of cGK.[58] In contrast, activation of BK_{Ca} channels by 17β-estradiol, corticosterone and dehydroepiandrosterone is β-subunit dependent.[59] The channel blocker paxalline has similar properties to iberiotoxin, in binding and occluding the extracellar pore.[57] Further investigation is needed to determine how BK_{Ca} channel function is maintained and regulated by several classes of endogenous compounds, including cyclic nucleotides.

Discussion on the limitations of this study should include an appraisal of the compounds selected for investigation, which do not all demonstrate high affinity for their target proteins. This characteristic is particularly evident for some NOS antagonists, which though not potent antagonists are isoform selective.[60] Although some of the investigated compounds fall short on specification, they represent the best of the compounds currently available. The study is also open to criticism on account of its unsophisticated approach to the study of molecular similarity. Drug development processes now requires the use of maximum computing power to test the activity of new compounds against a receptor protein or an array of molecular target panels. This retrospective and comparative evaluation of drug and hormone structures with established properties does not require the same degree of predictive computing power. As shown here, the methodology is capable of providing innovative data on relative molecular similarity within small-molecular-weight compounds.

In conclusion, the findings from this study demonstrate that the heterogeneous classes of ligand interacting with NOS and K^+ channels relate to substrate and nucleotide structures, respectively. Experimental observations of functional interaction between ligands specific for NOS, K_{ATP} and BK_{Ca} channels may be interpreted in terms of their structural similarity to regulatory purine nucleotides. The new evidence linking the acute modulation of BK_{Ca} channel function to similarity in steroid and nucleotide structure extends to gonadosteroids, glucocorticoids and mineralocorticoids.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

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