

# Asthma exacerbation by aspirin and chemical additives: use of a nucleotide template model to investigate potential mechanisms

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**Abstract.** Aspirin exacerbated asthma (AEA) affects approximately 10% of the asthmatic population. Clinical studies with a focus on abnormalities in arachidonate metabolism do not adequately account for susceptibility. Other pharmacological targets of aspirin receive less attention. Further investigation is required to elucidate mechanisms, improve on diagnosis and treatment. This study employs a molecular modeling approach, based on use of a nucleotide template, to standardise and compare molecular structures of compounds known to induce or prevent asthma. Results identify relative molecular similarity within the structures of drugs and cell mediators relevant to AEA and intolerance reactions. The investigated compounds provide equivalent fits to ligand structures for GABA, glycine, NMDA and nicotinic receptors. Chloride and ligand-gated ion channels are a common link between agents responsible for the induction and control of AEA. The methodology is applicable to compounds responsible for chemical-induced intolerance reactions.

**Key words:** Aspirin-induced asthma — Ion channels — Ligand-gated

## Introduction

The properties of aspirin are duplicitous in nature. The drug is of importance for anti-platelet and anti-inflammatory action and of interest in limiting the development of some neoplasms but potentially damaging to the physiology of the stomach, skin, nasal and bronchial passages, auditory and angio-neural systems (Mastalerz et al. 2005; Kwok and Loke 2010; Sheppard et al. 2014; Stevens et al. 2015). Assessments of prophylactic aspirin use in the general population focus on major risks of increased mortality and neglect the considerable potential for increased morbidity (Thorat and Cuzick 2015). Both harmful and therapeutic effects are attributed to the inhibitory action of aspirin on cyclooxygenase, the enzyme responsible for prostaglandin synthesis (Nandakishore et al. 2014). Altered arachidonate metabolism by aspirin also leads to the production of aspirin triggered lipoxin with strong inhibitory effects on inflammatory signaling pathways and their cytokines (Wang et al. 2011). A better understanding of the mechanism of aspirin sensitivity in asthma patients, a condition characterized by chronic

eosinophil inflammation and dysregulation of arachidonic acid metabolism, will facilitate development of a reliable *in vitro* test for better diagnosis of a potentially life-threatening condition (Velazquez and Teran 2013; Woessner 2017).

The molecular weight of aspirin is on a par with endogenous neurotransmitters and its chemical simplicity makes it multi-functional in regard to effects on enzymes, cell mediators and ion channels. In comparison to arachidonate metabolism, studies of salicylate action on ion channel function have received far less attention. Animal and *in vitro* studies demonstrate that aspirin and salicylate influence sodium, potassium, GABA<sub>A</sub>, glycine and NMDA ion channels (Lu et al. 2009). Hoang and co-authors (2010) are of the opinion that the primary defect in asthma is excitation-bronchoconstriction resulting from the stimulation of cell membrane ion channels and receptors.

Research into the mechanism of AEA is aided by studies of adverse reactions in different organ systems, investigations of asthma-inducing agents that do not belong to the category of non steroidal anti-inflammatories (NSAIDs) and the therapeutic efficacy of unconventional anti-asthma drugs such as cromolyn and furosemide. Furosemide normalizes increased lung epithelial permeability, even in severe asthma, and is effective against a wide range of asthma provoking agents, including aspirin (Myers et al. 1997; Bhure et al. 2009). The

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similar asthma prophylactic spectrum of furosemide and cromolyn is attributable to their effects on chloride transport (Alton et al. 1996; Alton and Norris 1996).

The majority of therapeutic drugs target G-protein controlled receptors (GPCR) *via* a critical guanine nucleotide structure at the focus of the biochemical process controlling cell membrane signal transduction events (Johnston and Siderovski 2007). The anti-asthma drugs targeting GPCR include adrenoceptor agonists, anticholinergic agents and leukotriene antagonists (IUPHAR database (2017), www.guidetopharmacology.org). Purine nucleotides also feature prominently in the regulation of ligand- and voltage-gated ion channels, which are pharmacologic targets of chromones and salicylates (Alton and Norris 1996; Li et al. 2013). Molecular similarity within nucleotide and hormone structures targeting GPCR and ion channels is relevant to the abnormalities in cell-signal transduction processes associated with asthma. Guanine nucleotide has proven to be a useful reference structure for the standardization and comparison of drug and hormone structures by means of a computational

chemistry program (Williams 2011). The methodology is applied here to the structures of compounds influencing aspirin intolerance and pseudo-allergic reactions, to determine if this approach assists in our understanding of the mechanisms responsible.

## Materials and Methods

Molecular formulae of the investigated compounds are listed in IUPHAR (www.guidetopharmacology.org) and Pubchem (<http://pubchem.ncbi.nlm.nih.gov>) databases. The representative agonist and antagonist compounds selected for investigation and literature sources linking ligand structures to ion channel and cell mediator receptor targets are given in Table 1.

The building of molecular structures, and conformational analysis to obtain minimum energy conformers for use in superimposition and fitting studies, is undertaken with the Nemesis software program (Oxford Molecular version

**Table 1.** Reference sources and targets of compounds under investigation

Compounds	Ion channels					Cell mediators		
	chloride channels			NMDA	nAch	Pg	LT	LXA4
	GABA <sub>A</sub>	glycine	other					
Furosemide	1	1, 9	11, 12	20	–	25	29	–
Cromolyn	–	–	11, 13	–	–	–	30	–
NPPB	2	–	14, 15	–	–	14	29	–
Niflumic acid	1, 2	–	2, 16	2,20	–	–	–	–
Aspirin /salicylate	3	10	–	21	–	26	31	–
Prostaglandin E2 (PgE2)	4	–	17	–	–	–	32	–
Isoguvacine	5	–	–	–	–	–	–	–
U93631	6	–	–	–	–	–	–	–
Bicuculline	5	7	–	–	–	–	–	–
Strychnine	7	7	–	–	–	–	–	–
Toluene-diisocyanate (TDI)	8	–	–	–	8	–	–	–
Leukotriene E4 (LTE4)	–	–	18	22	–	27	–	–
Lipoxin A4 (LXA4)	–	–	19	–	–	28	–	–
N-methyl-D-aspartate (NMDA)	–	–	–	23	–	–	–	–
Quinolinic acid	–	–	–	23	–	–	–	–
MK801	–	–	–	23	–	–	–	–
Nicotine	–	–	–	–	8	–	–	–
Epibatidine	–	–	–	–	8	–	–	–
Aspartame	–	–	–	24	–	–	–	–
Montelukast	–	–	–	–	–	–	–	33

1, Kumamoto and Murata 1997; 2, Zhao et al. 2016; 3, Gong et al. 2008; 4, Kimura et al. 1985; 5, Allan & Harris 1986; 6, Dillon et al. 1995; 7, Shirasaki et al. 1991; 8, Liu et al. 2006; 9, Dudeck et al. 2003; 10, Lu et al. 2009; 11, Alton et al. 1996; 12, Deisz et al. 2011; 13, Reinsprecht et al. 1992; 14, Breuer and Skorecki 1989; 15, Babot et al. 2005; 16, Liantonio et al. 2007; 17, Moeser et al. 2004; 18, Jett et al. 1991; 19, Verriere et al. 2012; 20, Lerma and Martin del Rio 1992; 21, Deng et al. 2013; 22, Kiyoyuki et al. 2015; 23, Pawley et al. 1996; 24, Abdollahi et al. 2001; 25, Pavord et al. 1995; 26, Nandakishore et al. 2014; 27, Salimi et al. 2017; 28, Kumar et al. 2014; 29, Perkins et al. 1992; 30, Yoshida et al. 1998; 31, Planaguma et al. 2002; 32, Narayanankutty et al. 2013; 33, Cai et al. 2007.

2.1). All ligand structures are minimum energy conformers in an uncharged form. The low energy conformer of GTP nucleotide is from a previous setting (Williams 2011). The program fits paired molecular structures on a 3 point basis. Fitting points comprise of selected atom pairs of similar type and partial charge within ligand and nucleotide structures. The sequence of fitting to the nucleotide is given for each compound in Table 2 (reading from left to right). The Nemesis program computes goodness-of-fit values, in respect of inter-atomic distance at each fitting point and the root mean square value. Ligand fitting points are identified in the Figures by colour-coded atoms: carbon-green, nitrogen-blue, oxygen-red. Bond order within molecular structures is not shown and the nucleotide structure is cropped to improve on presentation

**Table 2.** Fitting data for ligands superimposed on the GTP template

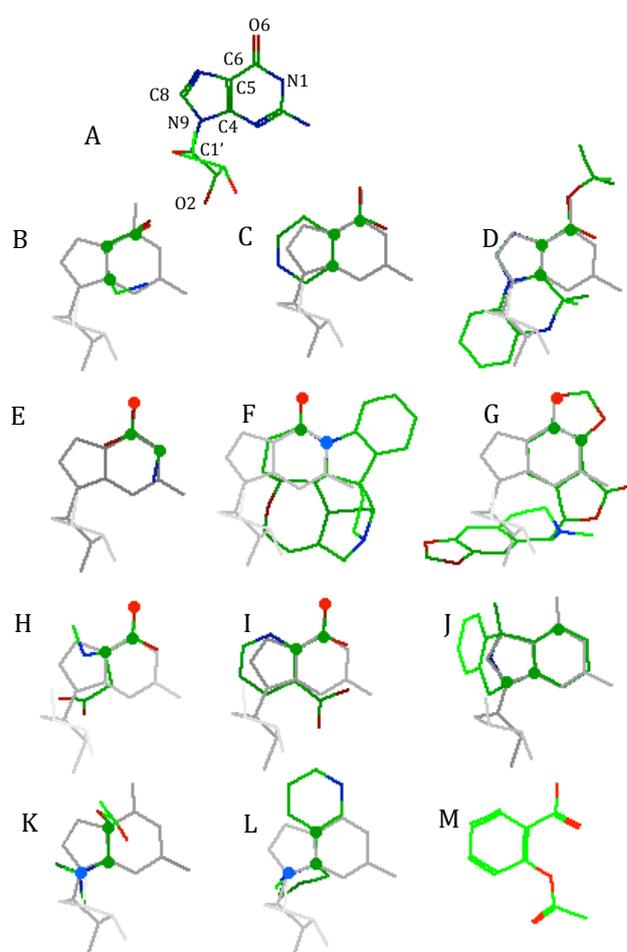
Compounds	Fitting points	Inter-atomic distance (Å)	RMS (Å)
Acetylcholine	N9C4C5	0.19,0.02,0.20	0.0277
Aspartame	O6C6C5	0.09,0.05,0.08	0.0034
Aspirin	C6C5C4	0.04,0.00,0.04	0.0041
Bicuculline	C4C5C6	0.01,0.03,0.01	0.0014
Cromolyn	O6C6C1'	0.10,0.12,0.08	0.0113
Epibatidine	C6C5C1'	0.06,0.01,0.07	0.0010
Furosemide	O6C6C5	0.07,0.03,0.04	0.0039
Furosemide	O6C6N1	0.09,0.02,0.08	0.0031
Furosemide	C6C5C4	0.02, 0.01,0.01	0.0019
GABA	C4C5C6	0.09,0.10,0.06	0.0156
Glycine	O6C6N1	0.02,0.06,0.08	0.0059
Isoguvacine	C4C5C6	0.01,0.02,0.02	0.0014
Lipoxin A4	C5C4O2	0.05,0.06,0.02	0.0084
LTE4	O6C6C5	0.01,0.04,0.05	0.0042
LTE4	C8N9C1'	0.07,0.13,0.06	0.0328
MK801	N9C4C6	0.11,0.06,0.05	0.0014
Montelukast	C8N9C1'	0.08,0.14,0.06	0.0337
Nicotine	N9C4C5	0.13,0.04,0.14	0.0052
Niflumic acid	C6C5C4	0.02,0.01,0.03	0.0012
Niflumic acid	O6C6C5	0.07,0.03,0.04	0.0036
NMDA	O6C6C5	0.09,0.03,0.09	0.0014
NPPB	C4C5C6	0.02,0.02,0.02	0.0025
Prostaglandin E2	C4C5C6	0.08,0.11,0.06	0.0212
Prostaglandin E2	C5C4C8	0.09,0.10,0.11	0.0006
Quinolinic acid	O6C6C5	0.07,0.03,0.05	0.0022
Salicylate	O6C6N1	0.03,0.03,0.06	0.0014
Strychnine	O6C6N1	0.01,0.01,0.02	0.0010
TDI	C6C5C1'	0.07,0.07,0.04	0.0134
U93631	C4C5C6	0.09,0.07,0.09	0.0286

LTE4, leukotriene E4; NMDA, N-methyl-D-aspartate; TDI, toluene-diisocyanate; NPPB, 5-nitro-2-(3-phenylpropylamino) benzoic acid.

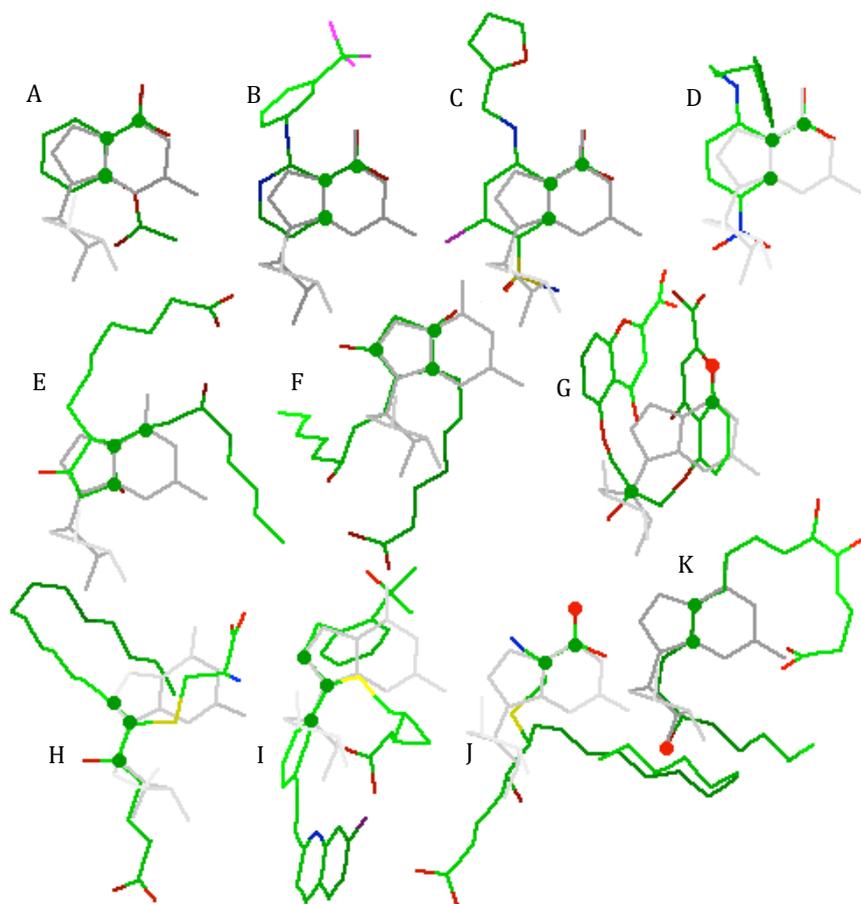
## Results

Results of the molecular modeling study are presented for ligand-gated ion channel (LGIC) ligands, and compounds modulating asthma and chemical-induced adverse reactions. Goodness-of-fit for the structures in Figures 1–3 does not exceed 0.20 Å and 0.0300 Å for inter-atomic distance and root mean square value, respectively (Table 2).

Compounds with a regulatory action on LGIC relate to several fitting patterns on the GTP template (Figure 1). The agonist and antagonist structures are ligands at GABA<sub>A</sub>, glycine, NMDA and nicotinic (nAch) receptors; bicuculline is active at glycine and GABA<sub>A</sub> receptors. GABA<sub>A</sub> and nAch ligands fit at the nucleotide endocyclic C4-C5 bond with the third fitting point at C6 (GABA) or N9 (nAch). The



**Figure 1.** Fits of ligand-gated ion channel ligands to guanine nucleotide (A) templates (grey): GABA (B), isoguvacine (C), U93631 (D), glycine (E), strychnine (F), bicuculline (G), NMDA (H), quinolinic acid (I), MK801 (J), acetylcholine (K), nicotine (L), aspirin (M). Agonist structures: B, C, E, H, I, K, L; antagonist structures: D, F, G, J.



**Figure 2.** Fits of asthma modulating agents to guanine nucleotide templates (grey): aspirin (A), niflumic acid (B), furosemide (C), NPPB (D), PGE2 (E), PGE2 (F), cromolyn (G), LTE4 (H), montelukast (I), LTE4 (J), LXA4 (K).

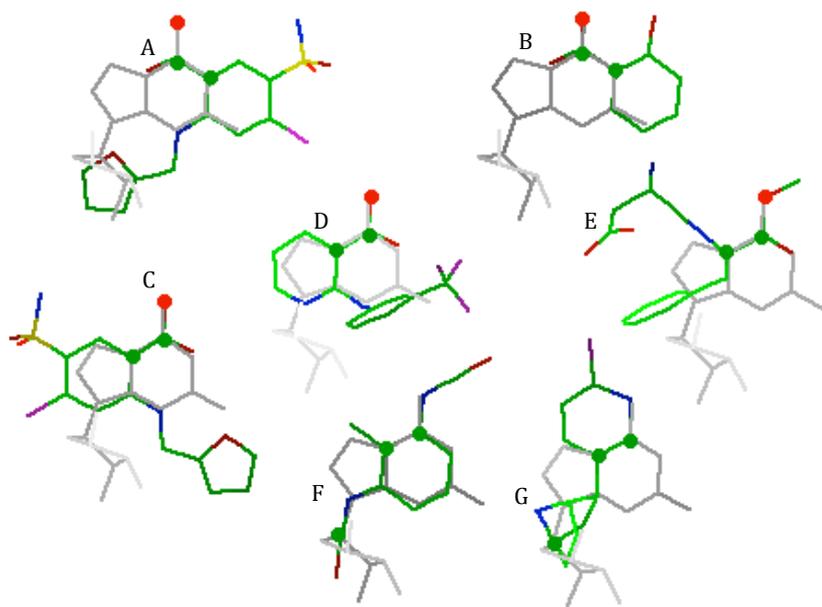
fits of glycine and NMDA ligands focus on the C6 carbonyl group of the guanine ring. Aspirin structure (M) is markedly similar to the GABA<sub>A</sub> agonist isoguvacine (C) and NMDA agonist quinolinic acid (I). The N9C4C5 fitting values of acetylcholine and nicotine, also replicated by minimum energy conformers of the agonists carbamoylcholine, RJR2429 and SIB1508Y (data not shown), are amongst the weakest of the LGIC ligands (Table 2).

Structures A–E, in Figure 2, provide a GABA<sub>A</sub>-like fit (C6C5C4) to the nucleotide template. Fitting values for structures B, C and D, chloride channel inhibitors with preventative effects on AEA, are better than the fit of GABA (Table 2). In comparison to the structure of aspirin (A), fitted chloride channel inhibitors present inhibitory alkyl chains and cyclic rings above the guanine ring in common with PGE2 (E) and cromolyn (G). The structures of cromolyn, LXA4 (K), LTE4 (H) and the LTE4 antagonist montelukast (I) have a fitting point on the ribose moiety of GTP at O2 or C1'. Structure J (LTE4) replicates the fit of NMDA in Figure 1 (the terminus of the side-chain of LTE4 comprises of a glutamate residue) with better fitting values than the alternative fit, template H. Template F provides an alternative to the GABA<sub>A</sub> fit of PGE2.

Figure 3 gives the glycine-like fits of furosemide (A) and salicylate (B) and the NMDA-like fits of furosemide (C), niflumic acid (D) and aspartame (E). Differences between antagonist (C and D) and exacerbator (E) action at the NMDA receptor are evident in the positioning of a non-fitting cyclic ring, which is absent in aspartame and NMDA (Figure 1) exacerbator structures. TDI (F) and the nicotinic ligand epibatidine (G) use the same fitting points on the GTP template but differ in the superimposition of a cyclic ring on the aminouracil moiety of the nucleotide.

## Discussion

The nucleotide template model facilitates the recognition of molecular similarity within the structures of compounds with established roles in the exacerbation and amelioration of asthma. In regard to ligand-gated ion channels, aspirin and salicylate provide NMDA-, GABA- and glycine-like fits to the nucleotide that relate to functionality at these receptors. Aspirin potentiates NMDA responses in ganglionic neuron cultures (Peng et al. 2003). Salicylate is equipotent at



**Figure 3.** Fits of intolerance-inducing agents and antagonists to guanine nucleotide templates (grey): furosemide (A), salicylate (B), furosemide (C), niflumic acid (D), aspartame (E), toluene diisocyanate (F), epibatidine (G).

GABA<sub>A</sub> and glycine receptors, inhibiting responses in neuron cultures from spinal dorsal horn and hippocampal slices, respectively (Gong et al. 2008; Lu et al. 2009). Salicylate has a pre-synaptic stimulatory effect on rat medullary inspiratory neurons, whereas a higher inhibitory concentration is antagonised by bicuculline, a GABA antagonist (Akada et al. 2003). GABA transmission is not confined to the central nervous system and release of the neurotransmitter from epithelial cells contributes to airway smooth muscle relaxation and regulation of mucous production (Danielsson et al. 2016). Glycine, calcium-activated chloride and Na-K-chloride co-transporter channels are also important in maintaining airways smooth muscle relaxation (Danielsson et al. 2014).

The structures of asthma ameliorating compounds such as cromolyn, PgE2 and furosemide use the same template fitting points as aspirin but reveal additional complexity in their fit to the nucleotide. These anti-asthma compounds influence chloride channel sites and demonstrate interaction *in vitro*. Furosemide and cromolyn attenuate challenge tests on asthmatic subjects and block conductance of a voltage- and calcium-dependent chloride channel in airways epithelium (Alton et al. 1996; Myers et al. 1997). Niflumic acid, a COX-2 inhibitor, targets the same inhibitory receptor site as furosemide at GABA<sub>A</sub> receptors (Zhao et al. 2016). Niflumic acid, NPPB and furosemide block chloride conductance, in airway epithelium, initiated by E-ring isoprostanes (Seto et al. 2008). Prostaglandins promote the action of GABA but their effects are also subject to regulation by ligands binding to the GABA<sub>A</sub> receptor (Kimura et al. 1985; Nonogaki et al. 1993). Furosemide

and NSAIDs both target specific sub-units of the GABA<sub>A</sub> receptor (Korpi et al. 2002; Sinkkonen et al. 2003).

The drugs under investigation are pluripotent in terms of receptor binding (see Table 1) and this is reflected in different receptor type fits to the guanine nucleotide template. Enhancement of the NMDA current by aspirin and salicylate is linked to the development of tinnitus and ganglionic cell death (Peng et al. 2003; Deng et al. 2013). Furosemide prevents NMDA receptor activation and inhibits the strychnine sensitive glycine receptor in cultured spinal cord neurons (Lerma and Martin del Rio 1992; Dudeck et al. 2003). Leukotrienes promote NMDA activity in neurons of the rat spinal cord (Kiyoyuki et al. 2015). There is experimental evidence for the participation of NMDA receptors in airway hyperreactivity but no established link between leukotrienes and NMDA receptors in airway inflammation (Antosova and Strapkova 2013; Anaparti et al. 2015).

Aspartame and TDI have records of long term controversial use in food and manufacturing processes. Aspartame causes neurological disturbances, activating the NMDA receptor and NO-cGMP pathway in mice (Abdollahi et al. 2001). The aspartame structure provides a NMDA-like fit to the nucleotide template. TDI has a considerable reputation for causing health problems, including asthma, in the work environment. *In vitro* studies demonstrate suppression and partial suppression of nicotinic and GABA receptor responses, respectively (Liu et al. 2006). TDI provides a similar nucleotide template fit to the nicotinic ligand epibatidine and is known to suppress a Ca<sup>2+</sup> current promoted by epibatidine in human neuroblastoma cells (Liu et al. 2006). Other irritant sensitising agents of interest in the occupational health

setting are organic acid anhydrides, which are structural analogues of aspirin with anti-inflammatory properties and inhibitory effects on prostaglandin synthesis (Killackey et al. 1984; Teng et al. 1987).

The multiple properties and concentration dependent effects of aspirin on cell targets contribute to the difficulty of establishing a mechanism for AEA. Aspirin targeting of neurotransmitter receptors may also provide opposing actions under different conditions. The global inhibitory action of GABA may change under depolarising conditions to contribute to glutamate excitotoxicity (Babot et al. 2005). Aspirin also influences the balance of cell mediators derived from the arachidonic cascade within the chemical milieu of inflammation. A singular focus on reciprocal changes in prostaglandin and leukotriene levels in AEA, therefore, inadequately addresses the action of aspirin in exacerbating asthma. Of the cell receptors implicated in AEA, chloride ion channels and NMDA receptors are common targets of aspirin, inflammatory mediators and the drugs providing protection.

In conclusion, molecular modelling methodology based on the use of a purine nucleotide template is a useful approach to the comparative study of asthma-inducing agents and anti-asthma drugs in respect of structure-activity data. Literature sources and molecular fitting data indicate that activated NMDA and chloride channels contribute to AEA and the condition does not result from protein targets entirely concerned with arachidonate metabolism.

**Conflict of interest.** I declare that there is no conflict of interest within the content of the manuscript. There are no sources of funding or sponsors associated with this work.

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Received: November 20, 2017

Final version accepted: January 17, 2018

First published online: June 29, 2018