Journal of Medicinal Chemistry

pubs.acs.org/jmc

Perspective

The Medicinal Chemistry of Caffeine

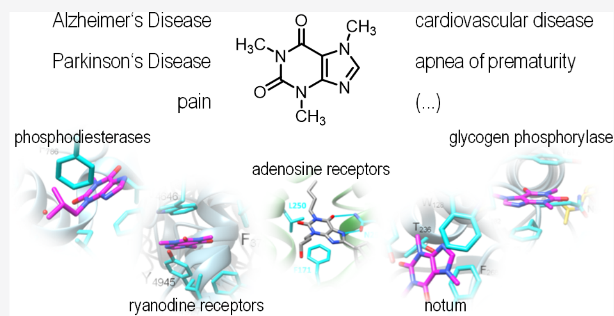
Giuseppe Faudone,[#] Silvia Arifi,[#] and Daniel Merk*

Cite This: J. Med. Chem. 2021, 64, 7156–7178

Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: The purine alkaloid caffeine is the most widely consumed psychostimulant drug in the world and has multiple beneficial pharmacological activities, for example, in neurodegenerative diseases. However, despite being an extensively studied bioactive natural product, the mechanistic understanding of caffeine's pharmacological effects is incomplete. While several molecular targets of caffeine such as adenosine receptors and phosphodiesterases have been known for decades and inspired numerous medicinal chemistry programs, new protein interactions of the xanthine are continuously discovered providing potentially improved pharmacological understanding and a molecular basis for future medicinal chemistry. In this Perspective, we gather knowledge on the confirmed protein interactions, structure activity relationship, and chemical biology of caffeine on well-known and upcoming targets. The diversity of caffeine's molecular activities on receptors and enzymes, many of which are abundant in the CNS, indicates a complex interplay of several mechanisms contributing to neuroprotective effects and highlights new targets as attractive subjects for drug discovery.



Downloaded via HIMACHAL PRADESH UNIV on August 11, 2021 at 01:03:16 (UTC).
See https://pubs.acs.org/sharingguidelines for options on how to legitimately share published articles.

1. INTRODUCTION

The purine alkaloid 1,3,7-trimethylxanthine, better known as caffeine (**1**, also termed theine, guaranine), is the most widely consumed psychostimulant drug in the world.[1] It acts as a central stimulant via some well-characterized mechanisms and also has several beneficial therapeutic effects some of which are not yet fully understood on a mechanistic level. Caffeine is naturally contained in seeds, nuts, and leaves of various plants and hence found not only in coffee beans but also, for example, in tea leaves, guarana berries, cocoa beans, and kola beans.

Caffeine consumption in the form of tea potentially dates back to 3000 BC in China, whereas evidence for cocoa bean use in ancient Mayan cultures as early as around 600 BC has been found.[2,3] Coffee consumption goes back to the 15th century in southern Arabia and northern Africa from where it came to Europe.[4] In 1819, caffeine was first isolated and termed "Kaffebase" by Friedlieb Ferdinand Runge, while the name caffeine was first used by Pierre-Joseph Pelletier.[4,5] Caffeine was later extracted also from tea under the name theine before it was proven to be the same molecule.[6,7] Hermann Emil Fischer was the first to synthesize caffeine in 1895 (Scheme 1) and to elucidate its molecular structure, which was part of his work awarded with the Nobel Prize in 1902.[8,9]

Despite many potential pathways to caffeine in plants, only one common caffeine biosynthesis route has been discovered but was found to be catalyzed by differentially specialized enzymes in different species (Scheme 2).[10] Caffeine biosynthesis originates from xanthosine which undergoes enzymatic N7-methylation and concomitant deribosylation to 7-methylxanthine. Another methylation step at N3 then produces theobromine (**2**) before enzymatic N1-methylation affords caffeine (**1**).[10] The natural occurrence of caffeine is known in several plant species where it serves as a defense mechanism to kill insects feeding on the plant through inhibition of insect phosphodiesterases, resulting in intracellular accumulation of cyclic adenosine monophosphate.[10−12]

For its stimulant activity, coffee had once been banned in various regions throughout history, for example, in Prussia, Sweden, and Mekka between the 16th and 18th century. Between 1984 and 2004, caffeine was a banned substance in sport competition for (well-documented) performance-enhancing effects but was removed from the World Anti-Doping Agency list in 2004.[13,14] Today, the caffeine content of beverages is restricted in some regions (e.g., to max. 0.02% or 200 ppm by the FDA), while caffeine as a powder is not regulated.

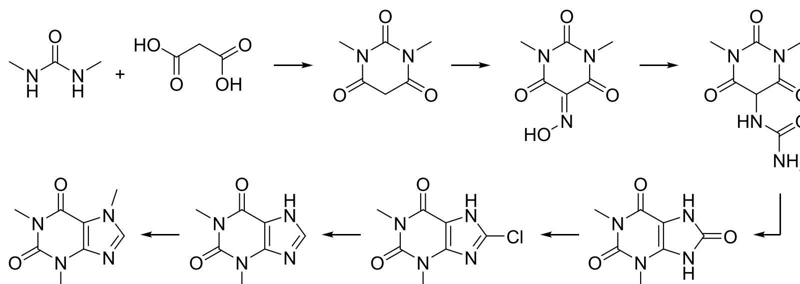
2. MEDICINAL CHEMISTRY OF CAFFEINE

Structurally, caffeine is closely related to the other natural xanthines theobromine (**2**), theophylline (**3**), and paraxanthine

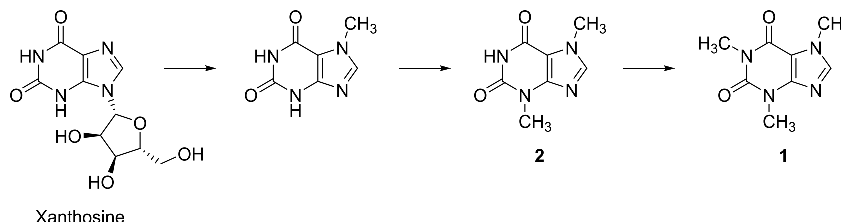
Received: February 9, 2021
Published: May 21, 2021

© 2021 The Authors. Published by American Chemical Society

Scheme 1. First Synthesis of Caffeine (1895) According to Fischer[8,9]

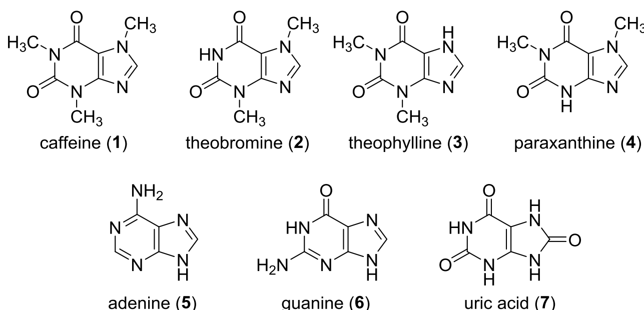


Scheme 2. Common Pathway of Caffeine Biosynthesis in Plants[10]



(4) and to many highly important endogenous (purine) molecules such as the purine bases adenine (5) and guanine (6) and the purine metabolite uric acid (7) (Scheme 3).

Scheme 3. Chemical Structures of Caffeine (1), Related Natural Xanthines 2–4, Purines 5 and 6, and Uric Acid (7)



The natural product (and approved drug) caffeine (1) is a very druglike molecule (Figure 1). It complies with the rule of

Property	Value
Melting point	235-237°C
Solubility (water)	21.7 g/L (~ 0.1 M), 66 g/L in boiling water
LogP	-0.07 – -0.01
Permeability (Caco2)	-4.41
pKa	14
HBD	0
HBA	3
PSA	58.44 Å²
rotatable bonds	0
Ro5 violations	0

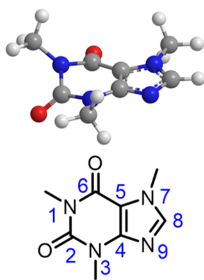


Figure 1. Properties, structure, and numbering of caffeine.

five,[15] is moderately lipophilic (experimental logP −0.01, other sources logP −0.07),[16−18] and can pass biological membranes/barriers. Caffeine (1) is well soluble in water (21.7 g/L) with pronounced temperature-dependent solubility (66 g/L in boiling water). All atoms of the two-ring skeleton are sp^2 hybridized, rendering the xanthine structure a flat scaffold (Figure 1).

Caffeine also has several roles as a drug. It is found (as caffeine citrate) on the WHO list of essential medicines for its respiratory stimulant action to be used in neonates.[19,20] Additionally, caffeine as a weak bronchodilator has been evaluated for asthma treatment, and a modest improvement of airway function in asthma patients has been observed for oral caffeine administration in a number of small clinical trials.[21,22] As an analgesic adjuvant, caffeine has considerable pharmacological relevance in multiple coformulations with nonsteroidal anti-inflammatory drugs (NSAIDs) for pain treatment. This use of caffeine in combination with mostly ibuprofen or paracetamol achieves a small but statistically significant improvement in pain relief.[23,24] It has been speculated that this effect is due to caffeine-mediated changes in bioavailability or pharmacokinetics of NSAIDs, but current evidence indicates that the analgesic adjuvant activity is mainly mediated through adenosine receptor (A_{2A}) antagonism.[25−29] In addition, several highly attractive effects of caffeine have been observed for example in the prevention of neurodegenerative diseases[30−32] and cancer immunotherapy,[33,34] many of which seem to be mainly mediated through effects on adenosinergic signaling (see chapter 3).

2.1. ADMET. The pharmacokinetics of caffeine were found to be affected by many factors, such as age, sex, hormone levels, liver health, obesity, smoking, and diets. The half-life, metabolism, and distribution of caffeine can hence vary significantly between individuals (Table 1). Owing to its moderate lipophilicity, caffeine can pass biological barriers well. After ingestion, it is rapidly and completely absorbed by the gastrointestinal tract reaching a bioavailability of up to 100%.[35] After intake of a high 5−8 mg/kg caffeine dose, peak plasma concentrations of up to 8−10 mg/L are reached after 0.5 to 2 h. Caffeine is an effective brain penetrant, and its stimulant effect arises after 45−60 min. The half-life of caffeine varies remarkably in healthy adults between 2.5 to a maximum of 12 h.[36,37] Caffeine is mainly metabolized in the liver by the cytochrome P450 (CYP) enzyme CYP1A2,[38] to paraxanthine (4, ~85%) and small amounts of theobromine (2, ~10%) and theophylline (3, < 5%).[36,39] In addition to the main CYP1A2-mediated pathway, caffeine can also be metabolized by

https://doi.org/10.1021/acs.jmedchem.1c00261
J. Med. Chem. 2021, 64, 7156−7178

Table 1. Pharmacokinetic Properties of Caffeine (in Adults)

absorption	
oral bioavailability	up to 100%
t_{max}	0.5−2 h
c_{max}	up to 8−10 mg/L
distribution	
volume of distribution	0.6 L/kg; brain penetrant
plasma protein binding	10%−36% (in vitro)
half-life	~5 h (varying)
metabolism	
metabolizing enzymes	CYP1A2, xanthine oxidase, N-acetyltransferase 2 (NAT2)
main metabolites	paraxanthine, (theobromine), (theophylline), 1-methylxanthine, 3,7-dimethyluric acid
elimination	
excretion	renal (metabolites), caffeine is reabsorbed in the tubulus
clearance	~0.078 L/kg/h (varying)

xanthine oxidase and N-acetyltransferase 2 (NAT2).[40] The main caffeine metabolite paraxanthine has similar pharmacodynamic activity on adenosine receptors as caffeine. Theophylline is slightly more active, while theobromine is markedly less active on adenosine receptors (K_i or IC_{50} > 100 μM) and, hence, has no stimulant activity.[34,41] The metabolites of caffeine are renally excreted, while caffeine itself is strongly reabsorbed in the renal tubulus.[42]

Large interindividual variability in CYP1A2 enzyme activity is the main reason for the remarkable variation in caffeine metabolism and pharmacokinetics.[43] CYP1A2 activity can be affected by endogenous factors such as genetic polymorphisms and hormone levels as well as exogenous contributions such as smoking, drug intake, and diet, resulting in the high variability of its enzyme activity.[44,45] Through polycyclic aromatic hydrocarbons which enhance liver enzyme activity, smoking causes CYP1A2 induction, which increases its metabolic rate by about 2-fold compared with nonsmokers.[46,47] A study with former smokers discovered that 3 weeks after smoking cessation, caffeine concentration reached a maximum value of 203% compared with caffeine consumers.[48] Unlike smoking, the use of oral contraceptives (such as lynestrenol, norgestrel, ethynodioldiac, levonorgestrel) may cause almost a doubling of caffeine concentration as hormone levels impact on liver enzyme activity.[36,49] Accordingly, caffeine metabolism is also reduced in pregnancy because of the decreased activity of CYP1A2, xanthine oxidase, and NAT2, and the half-life of caffeine can extend to as long as 12−18 h.[50,51] The slowed caffeine metabolism during pregnancy can also affect the unborn child.[52] Moreover, daily caffeine intake leads to caffeine accumulation in the placenta which may affect the fetus that cannot metabolize caffeine. After caffeine ingestion, caffeine enters the fetal circulation through the blood-placental barrier and neither the blood-placental barrier nor the fetus possess enzymes to break down caffeine leading to accumulation. Epidemiological studies have shown that caffeine consumption during pregnancy may be associated with intrauterine growth retardation, subfertility, and spontaneous abortion.[53−55] Intake of less than 300 mg of caffeine (per day) were considered safe for pregnant women's health,[38,56] but other results suggest that less than 300 mg caffeine per day can increase the risk for pregnancy failure.[55,57−59] Even intake of 100−200 mg of caffeine per day was found to increase the risk of miscarriage, fetal growth retardation, and low birth weight in some studies.[60−64] Moreover, treatment of pregnant rats with caffeine in drinking water (0.1, 0.3, and 1.0 g/L) to mimic coffee consumption decreased birth weight and affected fetal brain development with changes in neuronal gene expression leading to an increased number of neuronal cells. Hence, caffeine consumption in pregnancy has also effects on embryonal brain development in rodents, although the relevance of these findings in humans remains to be confirmed.[65]

Apart from these effects during pregnancy, caffeine has low toxicity and is generally considered safe.[66] An LD_{50} value of 192 mg/kg has been reported for rats, and 10−14 g (corresponding to about 150−200 mg/kg) is considered a lethal dose in humans.[67,68] Mild drug dependence to caffeine with withdrawal symptoms may develop with repeated consumption.[69] In the presence of certain pre-existing diseases such as epilepsy, the central stimulant caffeine may have severe adverse effects.[67,68,70]

As a substrate and inductor of CYP1A2,[71,72] caffeine can interfere with various drugs that are partially or entirely metabolized by this enzyme such as antidepressants, antipsychotics, and cardiovascular drugs. Although relevant interactions with caffeine are rare and restriction of caffeine consumption is not necessary in most cases,[73] there are also reports of pharmacologically relevant interference with caffeine.[74,75] For example, episodes of acute mental symptom exacerbations (such as anxiety, agitation, abdominal pain, a feeling of weakness, generalized stiffness, headaches, insomnia, intense paranoid ideation) were observed in a patient with heavy daily caffeine consumption (5−10 cups of coffee per day) under clozapine therapy. Upon discontinuing coffee consumption, these adverse events ceased.[75] A small study with seven schizophrenia patients demonstrated that clozapine concentrations decreased significantly after caffeine withdrawal. The greatest effect was observed in patients who were heavy smokers (40 cigarettes per day).[76] Concomitant caffeine and theophylline ingestion also reduced the elimination of both compounds.[77]

2.2. Molecular Activities of Caffeine.

Owing to its profound and very attractive pharmacological activities (see chapter 3) caffeine (**1**) has been extensively studied in multiple biological and biochemical assays with sometimes contradictory results. More than 300 pharmacological characteristics of caffeine are annotated in ChEMBL[78] (Table 2), some of which describe modulation of protein targets at millimolar concentrations that are not pharmacologically relevant, however. Moreover, caffeine has an aqueous solubility of approximately 100 mM and is very likely to aggregate and precipitate at concentrations above this threshold because of its planar architecture (Figure 1).

The best characterized molecular activity of caffeine is adenosine receptor antagonism of all subtypes with IC_{50} values in the range of 10 μM[34,79,80] (A_1R: 10.7 μM; A_{2A}R: 9.6 μM; A_{2B}R: 10.4 μM; A_3R: 13.3 μM; values from ref 34). Adenosine receptor antagonism is considered to mediate a majority of caffeine's known biological effects in human including the analgesic adjuvant activity and neuroprotective properties.[26−29] In addition, recent observations indicate the therapeutic potential of adenosine receptor antagonism in cancer immunotherapy.[33,34] Extensive studies have been conducted to elucidate the SAR of caffeine and derivatives on adenosine receptors and led to the development of highly potent

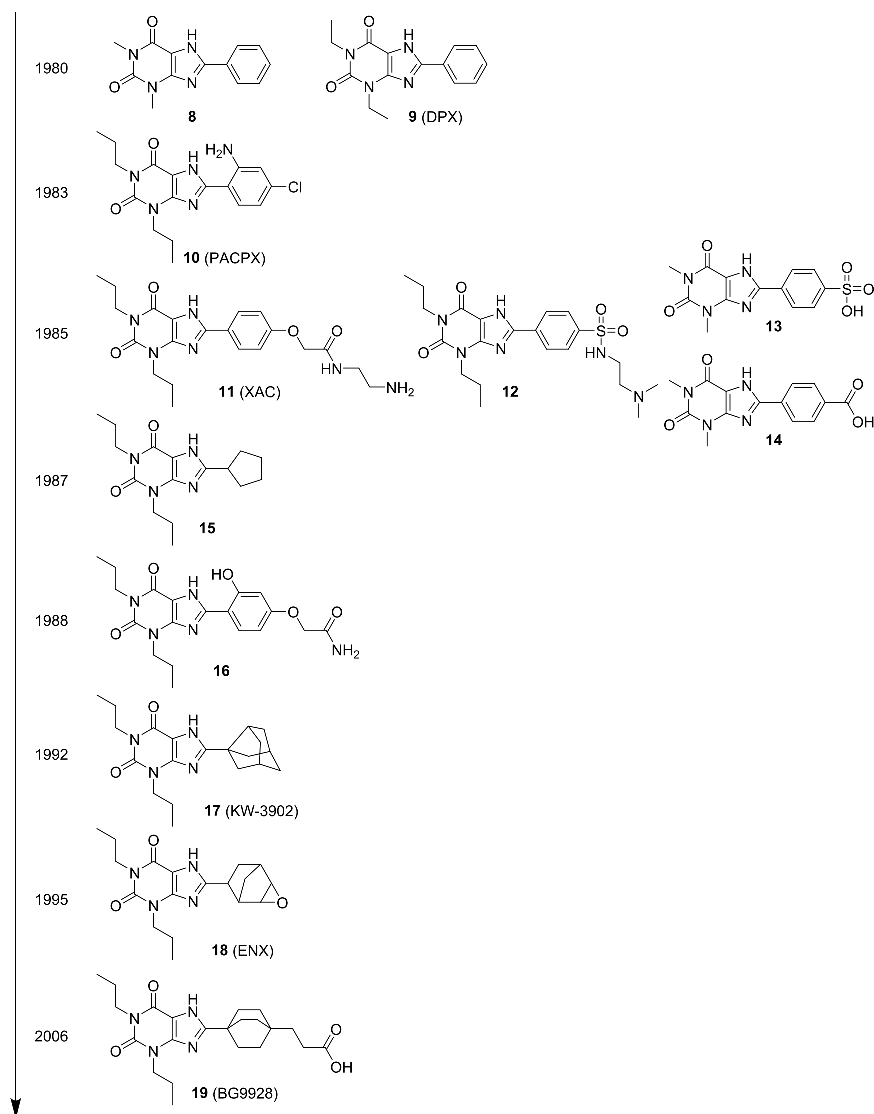
Table 2. Molecular Targets Tested for Caffeine as Annotated in ChEMBL or Reported in the Literature

molecular targets of caffeine (pEC_{50}, pIC_{50}, pK_i)	targets where caffeine was found to be inactive
confirmed targets	aldehyde dehydrogenase
acetylcholinesterase (pIC_{50} 5.1)	adrenergic receptors Alpha-2a, Alpha-2b, Beta-1, Beta-2, Beta-3
adenosine A1 receptor (pK_i 4.3−5.0)	angiotensin II type 2 (AT-2) receptor
adenosine A2a receptor (pK_i 4.3−5.6)	bradykinin B2 receptor
adenosine A2b receptor (pK_i 4.5−5.0)	cannabinoid CB1 receptor
adenosine A3 receptor (pK_i 4.9)	carbonic anhydrase II
glycogen phosporylase ($pK_i \sim 4$)	caspase-1
Notum (pIC_{50} 4−5)	cathepsin G
phosphodiesterases ($pIC_{50} < 4$)	C−C chemokine receptors type 2, type 4, type 5
ryanodine receptor ($pEC_{50} < 4$)	cholecystokinin A receptor
	COX-1, COX-2
targets with controversial results or pending confirmation	cysteinyl leukotriene receptor 1
bile salt export protein ($pIC_{50} < 4$)	CYPs 1A2, 2A6, 2B6, 2C19, 2C8, 2C9, 2D6,2E1
butyrylcholinesterase ($pIC_{50} < 4$)	dopamine receptors D1, D2, D4
CYPs 2C19, 3A4 (n.d.)	dopamine transporter
$GABA_A$ receptors ($pK_i < 4$)	endothelin receptor ET-A
guanine deaminase (pK_i 5.0)	estrogen receptors alpha, beta
HERG ($pIC_{50} < 4$)	glucocorticoid receptor
nicotinic acetylcholine receptors (n.d.)	histamine H1 receptor
PI3-kinase ($pIC_{50} \leq 4.1$)	HMG-CoA reductase
organic anion transporters 1B1, 1B3 (n.d.)	interleukin-8 receptor A, B
tailless homologue receptor (pIC_{50} 5.1)	leukocyte common antigen
	leukocyte elastase
	MAP kinases ERK1, ERK2, p38 alpha
	matrix metalloproteinases 1, 9
	melanocortin receptor 3, 4, 5
	monoamine oxidase A
	muscarinic acetylcholine receptors M1, M2, M3, M4, M5
	neurokinin 2 receptor
	neuropeptide Y receptor type 1
	norepinephrine transporter
	opioid receptors delta, kappa
	phosphodiesterase 5A
	receptor protein-tyrosine kinase erbB-2
	retinal dehydrogenase 2
	serine/threonine protein phosphatase 2B catalytic subunit, alpha
	serotonin 6 ($5\text{-}HT_6$) receptor
	sigma opioid receptor
	sodium channel alpha subunits; brain (types I, II, III)
	thromboxane-A synthase
	tyrosine-protein kinases FYN, LCK
	vasoactive intestinal polypeptide receptor 1
	vasopressin V1a receptor

adenosine receptor ligands (see chapter 2.2.2). Weak inhibition of phosphodiesterases (PDE) is another long known activity of caffeine which has initiated elaborate medicinal chemistry with xanthines as a natural template for the development of potent PDE inhibitors[81−84] (see chapter 2.2.3). In addition, caffeine was found to modulate $GABA_A$ receptors through the benzodiazepine binding site[85−87] and to inhibit acetylcholinesterase[88−91] with intermediate to high micromolar potencies which may be involved in CNS effects of caffeine, as well. Inhibition of butyrylcholinesterase by caffeine has been speculated, too, but is controversial.[90] A recent study has also reported modulation of nicotinic acetylcholine receptors by caffeine in an allosteric fashion.[88] Reports suggesting involvement of phosphatidylinositol 3-kinase (PI3K) inhibition by caffeine in its biological/pharmacological activities require further attention and validation.[92−94] Caffeine also is an activating ligand of ryanodine receptors (RyR) which are intracellular Ca^{2+}-release channels and essentially important for muscle and neuronal function.[95,96] While this activity of caffeine has been described decades ago and was only observed at very high concentrations (max. efficacy at ≥10 mM), three recent studies[97−99] have succeeded in providing a structural basis for RyR modulation by caffeine and confirmed binding sites for caffeine in the pore region, through which it modulates the channel open probability (see chapter 2.2.4).

Caffeine has also been speculated as weak inhibitor of the transmembrane transporters bile salt export protein, organic anion transporter 1B1 and organic anion transporter 1B3, as well as the cytochrome P450 enzymes 2C19 and 3A4.[100−102] While these weak activities likely have few pharmacodynamic consequences per se, they can affect the pharmacokinetics of other drugs. Another metabolic enzyme for which an

Scheme 4. Selected Xanthine-Derived A_1 Antagonists



interaction with caffeine was reported is guanine deaminase which was inhibited in vitro by caffeine at intermediate micromolar concentrations.[103] In vitro studies have additionally observed a direct inhibition of the human Ether-a-go-go-Related Gene (hERG) potassium channel by caffeine through stabilization of an open state,[104] but the tested concentrations were extremely high (5 and 20 mM); however, no effect was observed at relevant concentrations.

An allosteric inhibitory activity of caffeine has also been discovered on glycogen phosphorylase, the rate-limiting enzyme in glucose release from (hepatic) glycogen storages.[105,106] Glycogen phosphorylase has received considerable attention as a potential target for antidiabetic therapy, but interest has faded.[107] Biochemical and biophysical (SPR) studies have determined a potency/affinity of approximately 100 μM for caffeine in inhibiting glycogen phosphorylase, and cocrystal structures have confirmed and characterized caffeine binding to the enzyme (see chapter 2.2.5).[105,108] Recent findings characterize caffeine as inhibitor of the Wnt deacylase Notum which acts as negative feedback regulator of Wnt signaling (see chapter 2.2.6).[109,110] Among a number of activities reported so far, Notum appears to be involved in cancer and in neuronal function.[111−114] Very recently, we have discovered modulation of the brain nuclear receptor tailless homologue (TLX) by caffeine and derivatives which may emerge as another protein target involved in the neuroprotective properties of caffeine.

In addition to these proteins for which a direct modulation by caffeine has been demonstrated or at least suggested, caffeine was profiled on multiple further molecular targets and found to be inactive (Table 2).

2.2.1. Macromolecular Complex Structures with Bound Caffeine. As of today, the protein data bank contains 27 macromolecular structures of human proteins with caffeine as ligand. Among them, two structures are stabilized adenosine A_{2A}R receptors complexed with caffeine (pdb IDs 3RFM, 5MPZ), 19 entries (pdb IDs 5T15, 5T9S, 5T9R, 5T9 V, 5T9M, 5T9N, 5TA3, 5TAZ, 5TAS, 5TB4, 6JI0, 6JII, 6JI8, 6JIU, 6JIY, 6JRS, 6JRR, 6JV2, 6JHN) refer to ryanodine receptors with bound caffeine, one structure (pdb ID 6TV4) presents the Notum complex with caffeine bound to the catalytic pocket, and five entries (pdb IDs 3DDS, 3DDW, 3DD1, 1L7X, 1L5Q) refer to glycogen phosphorylase with caffeine bound to an allosteric inhibitory site.

https://doi.org/10.1021/acs.jmedchem.1c00261
J. Med. Chem. 2021, 64, 7156−7178

2.2.2. Caffeine as Adenosine Receptor Ligand. Adenosine receptor (ADOR) antagonism—from a medicinal chemistry point of view—is the most intensively studied biochemical activity of caffeine (**1**). The SAR of the natural product has been evaluated on all ADOR subtypes and caffeine has served as a lead compound for multiple potent and selective ADOR antagonists, some of which have high relevance as tool compounds and as drugs. The SAR and in vitro characterization data of caffeine and descendants as ADOR antagonists are complex and in some cases contradictory, which is partly due to the use of diverse in vitro test system types (radioligand binding, cellular assay, etc.), species differences (human, mouse, guinea pig, rat, etc.),[115] and the belated discovery of further adenosine receptors leading to a retrospective reevaluation of potency and selectivity profiles. Medicinal chemistry efforts on ADOR antagonist development and optimization have been immense and have also created several chemotypes with little structural relation to xanthines remaining. The comprehensive SAR and developmental history of ADOR antagonists has been summarized in several reviews[34,116−121] and will not be the topic of this Perspective. However, in the following paragraphs, we provide an overview of the SAR of xanthines as ADOR antagonists and highlight milestone compounds without comprehensively describing the SAR in detail.

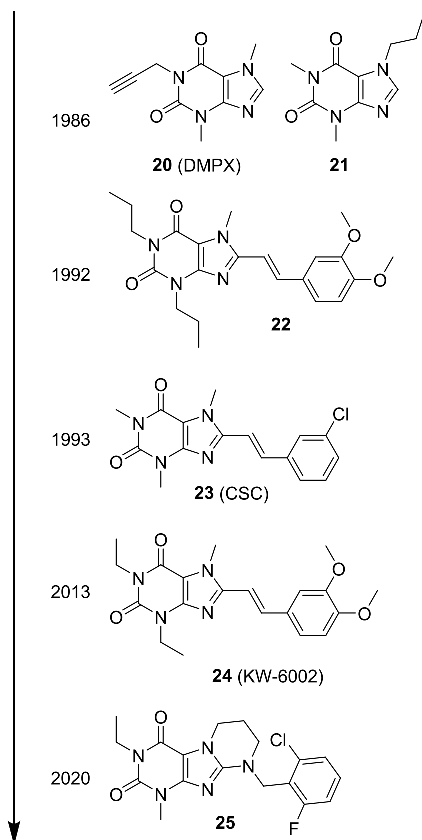
Shimizu et al.[122] reported the first evidence that caffeine affects cAMP generation in guinea pig cerebral cortex, which may occur through the modulation of ADORs. As G-protein coupled receptors (GPCRs), the ADORs consist typically of seven transmembrane helices which recognize small molecules on the extracellular surfaces and translate the ligand signal via G-protein mediated coupling into intracellular effects such as the regulation of intracellular cAMP levels via adenylate cyclase.[123,124] Four subtypes of ADORs have been identified to date, namely, A_1 (uniprot: P30542), A_{2A} (uniprot: P29274), A_{2B} (uniprot: P29275), and A_3 (uniprot: P33765).[125] A_1 and A_3 are coupled to $G_{i/o}$ and therefore inhibit adenylate cyclase, leading to low cAMP levels, low protein kinase A (PKA) activity, and decreased subsequent phosphorylation of the transcriptional activator cyclic AMP response element binding protein (CREBP). A_{2A} and A_{2B} are coupled to G_s promoting adenylate cyclase activity, resulting in elevated cAMP levels and higher PKA activity and also to G_q leading to activation of phospholipase C (PLC) with a subsequent enhancement of protein kinase C (PKC) activity and elevated intracellular Ca^{2+} levels.[126−128]

Extensive medicinal chemistry efforts have been made over the last decades to elucidate the SAR of caffeine and analogues on ADORs involving variations on almost all positions of caffeine. Early stages have focused predominantly on the easily accessible positions 1, 3, 7, and 8 by varying the alkylation patterns at position 1, 3, and 7 and by introducing 8-aryl- and cycloalkyl residues. Early studies on modifications in 7 position indicated that no polar substituents were tolerated, and only minor improvements in potency were achieved by extending the 7-methyl to propyl, allyl or propargyl motifs.[129] Therefore, SAR elucidation mainly focused on the remaining 1- and 3-positions followed by extensive evaluation of the favored 8-phenyl moiety before further/alternative expansion of caffeine at position 8 was studied. Considerable work has also focused on improving aqueous solubility of the compound class.

2.2.2.1. A_1 Receptor. Early studies report caffeine and theophylline as nonselective (A_1/A_2) antagonists with affinities in the 5−200 μM range depending on species and tissues.[130] First, remarkable improvements toward low micromolar affinity were achieved by introduction of a phenyl residue at the 8-position of theophylline resulting in 8-phenyltheophylline (**8**, IC_{50} 0.5 μM, Scheme 4) and 1,3-diethyl-8-phenylxanthine (**9**, IC_{50} 1 μM).[130] Only three years later, Bruns et al. reported PACPX (**10**, 1,3-dipropyl-8-(2-amino-4-chlorophenyl)xanthine) with a K_i value as low as 22 pM corresponding to a factor of 70.000 improvement in potency.[131] In a functionalized congener approach of 1,3-dipropyl-8-phenylxanthine, Jacobson et al. discovered further highly active xanthine derivatives with nanomolar affinities for A_1 and for A_2 including XAC (**11**,*N*-(2-aminoethyl)-2-[4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1*H*-purin-8-yl)-phenoxy]-acetamide), which was also used as an A_1-selective radioligand in subsequent studies because of its high affinity (0.17−3 nM depending on species).[132,133] The early SAR results demonstrated that larger linear N-propyl substituents at positions 1 and 3 as well as introduction of an 8-phenyl moiety were favored for A_1 receptor affinity and further potent ligands complying with this pattern were developed (**12**−**14**, **16**). The 8-phenyl substituent was soon successfully replaced with cycloalkyl moieties (**15**, **17**−**19**) leading to potent and selective A_1 receptor antagonists with DPCPX (**15**,8-cyclopentyl-1,3-dipropylxanthine, K_i 0.46 nM, A_2/A_1 740-fold selectivity),[134] KW-3902 (**17**,1,3-dipropyl-8-(3-noradamantyl)-xanthine, K_i 1.3 nM, A_2/A_1 290-fold selectivity),[135] and ENX (**18**,1,3-dipropyI-8 [2-(5,6-epoxy)norbornyI]xanthine, K_i 0.95 nM, A_2/A_1 400-fold selectivity)[136] as most notable examples. Simultaneously, the limited aqueous solubility of xanthine-based ADOR antagonists was addressed as a prerequisite for further consideration of the compound class in therapeutic applications. The first potent caffeine derived ADOR antagonist **8** comprised a low aqueous solubility <10 μM. Early improvements were achieved by introducing polar, ionizable groups such as *p*-sulfophenyl (**13**, aqueous solubility >20 mM, K_i 4.5 μM, nonselective) and *p*-carboxyphenyl (**14**, aqueous solubility 90 μM, K_i 3.0 μM, nonselective), which also caused a significant loss in potency on ADORs, however.[137] A quantitative structure activity relationship (QSAR) approach succeeded in retaining high ADOR affinity and enhanced aqueous solubility with further 1,3-dialkyl-8-*p*-sulfonamide-theophyllines such as **12** (K_i 6.5 nM, aqueous solubility 19.9 mg/mL = 43 mM).[138] Similarly, Daly et al. extended the *para* substituent of the 8-phenyl residue to obtain 1,3-dipropyl-2-hydroxy-4-[(carbamoylmethyl)oxy]phenylxanthine (**16**, K_i 6.5 nM, aqueous solubility 5.6 mM, A_2/A_1 190-fold selective).[139] Kiesman et al. prepared a set of 1,3-substituted 8-cyclohexyl- and 8-bicyclo-[2.2.2]octylxanthines and discovered BG9928 (**19**,3-[4-(2,6-dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-bicyclo[2.2.2]oct-1-yl]-propionic acid, K_i 7.4 nM, aqueous solubility 59 mM, A_{2A}/A_1 915-fold selective, A_{2B}/A_1 12-fold selective) as a potent and selective A_1 antagonist with preferable bioavailability profile.[140]

2.2.2.2. A_{2A} Receptor. A first tendency toward A_2 selective xanthine derivatives has been reported by Ukena et al. with DMPX (**20**, 3,7-dimethyl-1-propargylxanthine) and 7-propyl-1,3-dimethylxanthine (**21**) as A_2 receptor antagonists with an A_1/A_2 selectivity ratio of 10−20 (Scheme 5).[141] DMPX also demonstrated improved in vivo efficacy against 5′-*N*-ethylcarboxamidoadenosine (NECA)-induced hyperthermia compared with caffeine.[142] Further improvement in A_2 selectivity resulted from 8-cycloalkyl (cyclopentyl and cyclohexyl)

Scheme 5. Selected Xanthine-Derived A_{2A} Antagonists



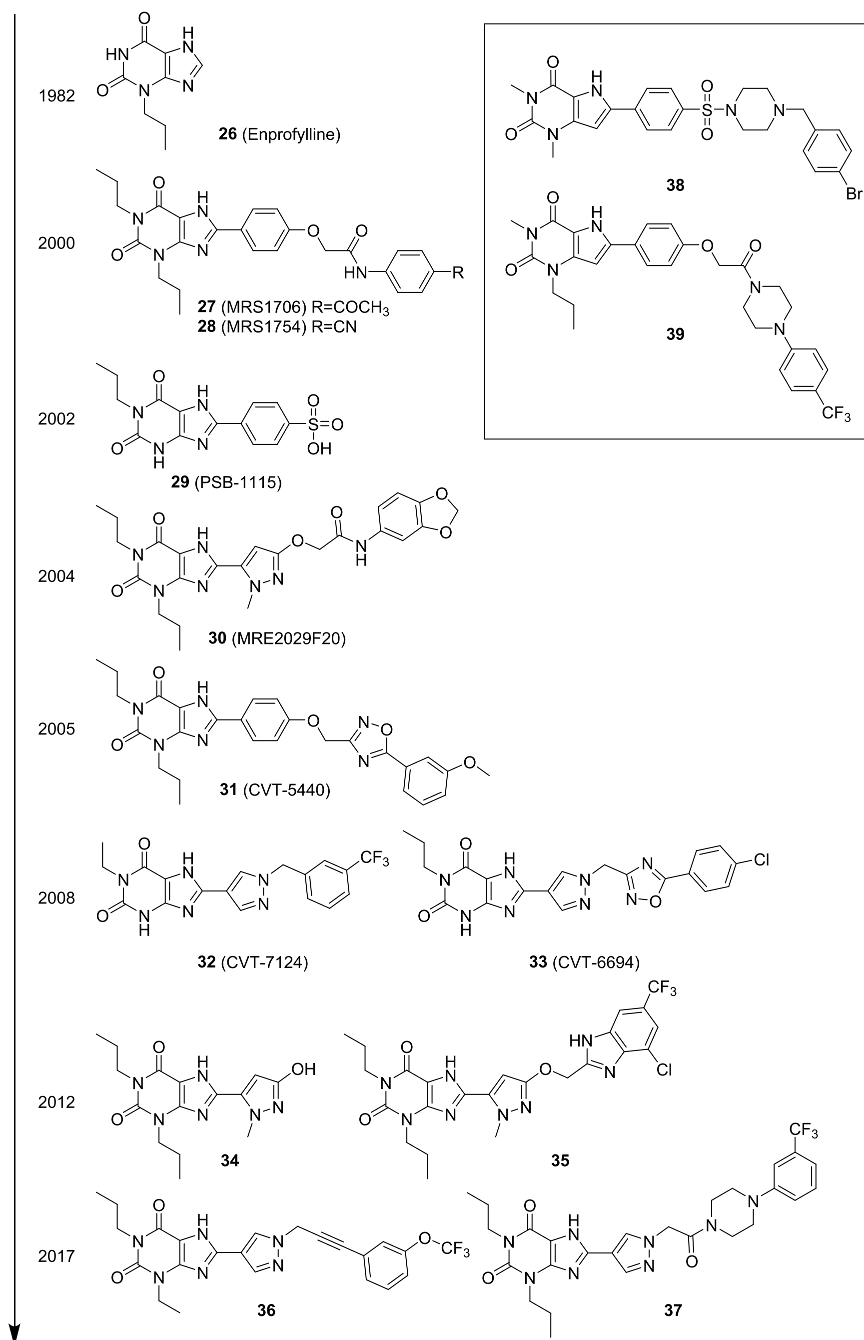
caffeine derivatives, which seemed contradictory at a first glance since 8-cycloalkyltheophyllines were predominantly found as A_1-selective.[143] Alternatively, an (*E*)-3,4-dimethoxystyryl group at position 8 of 1,3-dialkyl-7-methylxanthines (**22**) produced high potency and selectivity toward A_2, but aqueous solubility was poor (<10 μg/mL).[144,145] Further SAR elucidation of 8-styryl-1,3,7-alkylxanthines by Jacobson et al. revealed meta substitution on the styryl ring as crucial for potency and selectivity.[146] CSC (**23**, 8-chlorostyrylcaffeine, K_i 54 nM, A_1/A_2 520-fold selectivity) emerged as a first potent tool compound for A_2.[147] Despite having concomitant monoamine oxidase B (MAO-B) inhibitory potency (∼100 nM),[148] CSC served as lead structure for the development of KW-6002 (**24**, Istradefylline, (*E*)-1,3-diethyl-8-(3,4-dimethoxystyryl)-7-methylxanthine), which has received considerable attention for its high potency and selectivity toward A_{2A}. It is approved in Japan[149] and the United States (trade name Nourianz)[150] to treat "off time" in Parkinson's Disease. Very recent studies have reported novel pyrimido[2,1-*f*]purinedione based A_2 antagonists as exemplified by **25** (K_i 264 nM), which also act as monoamine oxidase B (MAO-B) inhibitors.[151,152]

2.2.2.3. A_{2B} Receptor. Enprofylline (**26**, 3-propylxanthine, Scheme 6) was reported in 1982 as the first xanthine comprising preference for A_{2B}[153,154] but it took until 2000 before the potent caffeine-based A_{2B} antagonists MRS 1706 (**27**, K_i 1.39 nM), MRS 1754 (**28**, K_i 1.97 nM),[155] and PSB-1115 (**29**, K_i 53.4 nM) derived from XAC (**11**) were described. The latter example lacks the common 3-propyl substituent and displays favorable aqueous solubility because of its 8-*p*-sulfophenyl moiety.[156] Furthermore, bioisosteric replacement of the amide linker of XAC (**11**) with an 1,2,4-oxadiazole yielded the potent and highly A_{2B} selective antagonist CVT-5540 (**31**, K_i 50 nM, A_1/A_{2B} > 200, A_{2A}/A_{2B} > 200 and A_3/A_{2B} > 167-fold selectivity).[157] Concomitantly, Baraldi et al. reported a large series of xanthines comprising a heterocycle in 8-position thereby introducing the *N*-methylpyrazole as structural feature that was later incorporated in several other ADOR antagonists. MRE2029F20 (**30**, K_i 5.5 nM) was the most active compound of the series.[158] Later examples of 8-pyrazolo-1,3-dialkylxanthines include **32**-**35**.[159−162] **34** (K_i 4 nM, A_1/A_{2B} 183 fold selectivity, A_{2A},A_3/A_{2B} > 250 fold selectivity) and **35** (K_i 9.4 nM, A_1/A_{2B} 269 fold selectivity, A_{2A},A_3/A_{2B} > 106 fold selectivity) were obtained by using a comparative molecular field analysis approach (CoMFA) based on 52 8-pyrazoyl xanthines with known biological activity to predict several potent and selective derivatives.[162] Recent progress in xanthine based A_{2B} antagonists has been made in the linker region of the 8-substituent by replacing the previous amide linkers with a linear 1-prop-2-ynyl moiety (**36**, K_i 13 nM)[163] or with heterocyclic groups (e.g., pyrrolidinone and piperazine, **37**, K_i 4.5 nM). The latter heterocyclic modifications also provided advantages regarding pharmacokinetics with a bioavailability of 65% for **37** in mice.[164]

Another notable series of caffeine inspired A_{2B} antagonists is based on a 9-deazaxanthine scaffold commonly equipped with an 8-phenyl residue that is further extended with a piperazine or piperidine linked aromatic tail. Several highly potent and selective A_{2B} antagonists of this chemotype have been developed such as **38** (K_i 1 nM, A_1/A_{2B} 183-fold selectivity, A_3/A_{2B} 12660-fold selectivity) and **39** (K_i 2 nM, A_1/A_{2B} 44-fold selectivity, A_{2A}/A_{2B} 159-fold selectivity, A_3/A_{2B} 180-fold selectivity).[165,166]

2.2.2.4. A_3 Receptor. Compared to A_1 and A_2 receptors, less SAR knowledge is available on xanthines as A_3 receptor antagonists (Scheme 7). I-ABOPX (**40**, 3-(3-iodo-4-aminobenzyl)-8-(4-oxyacetate)phenyl-1-propylxanthine, K_i 18 nM)[167] was one of the first potent A_3 antagonist tool compounds, and N7-ribosylated 1,3-dibutylxanthine was another early reported but rather weak ADOR antagonist (6 μM) with a slight A_3 preference.[168] Its subsequent structural optimization produced the more potent and selective analogue **41** (K_i 229 nM, A_1/A_3 160-fold selective and A_{2A}/A_3 > 400-fold selective) comprising a 5-*N*-carboxamidoriboside at position 7.[169] Later xanthine inspired A_3 antagonist generations are based on tricyclic scaffolds such as pyrido[2,1-*f*]purine-2,4-diones and imidazo[2,1-*f*]purine-2,4-diones incorporating the former xanthine moiety. **42** (K_i 4.0 nM)[170] and **45** (K_i 0.8 nM, selectivity over A_1 and A_2 ≫ 1250)[171] are highly optimized selective A_3 antagonists of these series. Imidazo[2,1-*i*]purin-5-ones are another noteworthy class of xanthine-derived A_3 antagonists. Because of an additional basic nitrogen, potent A_3 antagonist examples of this series such as PSB-11 (**43**, K_i 2.3 nM, A_1/A_3 190-fold selective, A_2/A_3 > 900-fold selective)[172] and PSB-10 (**44**, K_i 0.433 nM, selectivity over A_1 and A_2 > 800)[173] provide improved aqueous solubility. Important progress in A_3 antagonist development has lately been achieved with the pyrido[2,1-*f*]purine-2,4-dione skeleton of **42** by studying the residence time (RT) to elucidate structure−kinetics relationships (SKR). **46** (K_i 0.38 nM, RT = 376 min)[174] emerged as a valuable lead compound with a long RT which is considered as an important feature for in vivo efficacy. Moreover, **47** (K_i 27 nM)[175] comprising a fluorosulfonyl warhead attached to the xanthine N-3 via a flexible linker, has recently been reported as a covalent A_3

Scheme 6. Selected Xanthine-Derived A_{2B} Antagonists. Inset Shows 9-Deazaxanthines



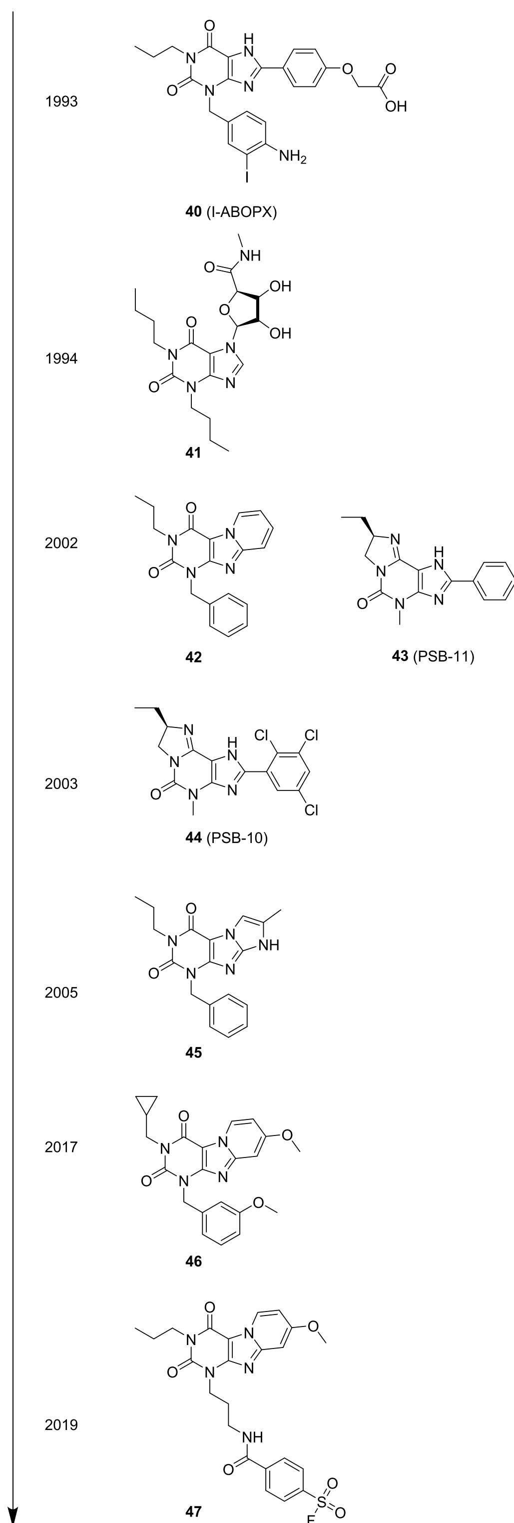
antagonist. It was designed to form a covalent bond with Tyr265 of the A_3 receptor through a nucleophilic substitution at the sulfonyl group which was confirmed by site directed mutagenesis.

2.2.2.5. Xanthine Bound Adenosine Receptor Structures. From the structural biology point of view, A_{2A} is the most elucidated adenosine receptor subtype with more than 50 solved cocrystal structures, five of which comprise a bound caffeine derivative as ligand (pdb IDs 3REY, 3RFM, 5MZJ, 5MZP, 5N2R). In addition, a cocrystal structure of A_1 complexed with a caffeine derivative has been solved (pdb ID 5N2S). The xanthine derivative PSB36 has been cocrystallized with both the A_1 and A_{2A} subtypes and hence provides a valuable basis for comparative evaluation of the ligand—receptor interactions.[176,177] Overall, the caffeine binding sites of A_1 and A_{2A} receptors are very similar. In the A_1 receptor binding site, the xanthine scaffold participates in a hydrophobic contact with Leu250 and in π-stacking with Phe171. In addition, the 6-carbonyl oxygen and the 7-nitrogen of the xanthine core form hydrogen bonds to the Asn254 side chain (Figure 2). This binding mode of the basic xanthine scaffold is conserved in other xanthine bound ADOR cocrystal structures. Interestingly, despite forming one hydrogen bond less to Asn254 due to the N7 methyl substituent, caffeine has comparable adenosine receptor affinity as theophylline.[176]

Compared with A_1, the A_{2A} receptor binding site is narrow, which affects the accommodation of bulky alkyl substituents on N1 and N3. Therefore, PSB36 is shifted deeper into the

https://doi.org/10.1021/acs.jmedchem.1c00261
J. Med. Chem. 2021, 64, 7156−7178

Scheme 7. Selected Xanthine-Derived A_3 Antagonists



binding cavity in A_1 than in A_{2A}. Steric hindrance by Met270 in A_{2A} toward the 8-noradamantyl substituent provides further structural explanation for the accommodation of bulkier ligands by A_1 bearing Thr270 at this position (Figure 3). Mutagenesis studies have confirmed this assumption.[176]

2.2.3. Caffeine as Phosphodiesterase Inhibitor. Inhibition of cyclic nucleotide phosphodiesterases (PDE) also was one of the first biological activities of caffeine to be discovered.[178,179]

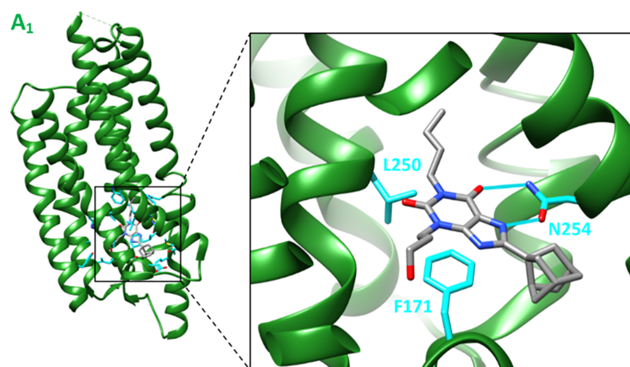


Figure 2. PSB36 bound to the A_1 receptor (pdb ID 5N2S). Interacting amino acids are highlighted.

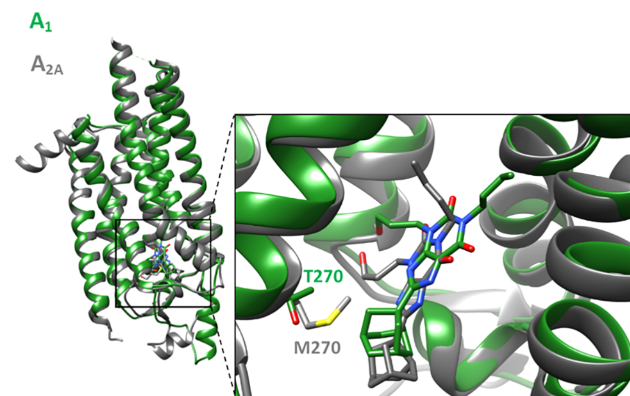


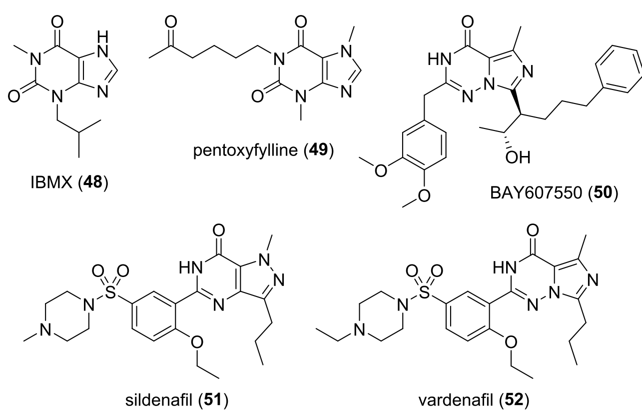
Figure 3. Superimposed structures of A_1 (green, pdb ID 5N2S) and A_{2A} (gray, pdb ID 5N2R) receptor in complex with PSB36. The bulkier Met270 in A_{2A} affects binding of bulky 8-substituents, while Thr270 of A_1 provides more space to ligands.

It has inspired vast efforts of medicinal chemistry in PDE inhibitor design, leading to the development of potent and selective inhibitors of the 11 PDE isoenzymes in humans. The majority of PDE inhibitors comprises a two-ring scaffold to mimic the purine respectively xanthine structure of PDE substrates and early inhibitors. Caffeine and theophylline were found to inhibit most human PDEs with weak potency in the high micromolar range. After the discovery of caffeine and the related xanthines theophylline and theobromine inhibiting PDEs, multiple alkylxanthines were developed to optimize potency and selectivity.[178] 3-Isobutyl-1-methylxanthine (IBMX, **48**) was the first widely used nonspecific PDE inhibitor that blocks all PDEs except PDE8, PDE9, and PDE11 at low micromolar potency.[180] Particularly (bulky) substituents in positions 2 and 9 of the underlying purine scaffold were characterized as major switches to tune potency and selectivity toward some PDEs (PDE1, PDE2, PDE4, and PDE5), while among other PDEs, scaffolds have evolved that markedly differ from caffeine/xanthines.[178,181,182] Notable PDE inhibitors comprising structural elements of caffeine are (Scheme 8), for example, pentoxyfylline (**49**) as a weak nonselective PDE inhibitor, the selective PDE2 inhibitor BAY607550[183] (**50**, IC_{50} (PDE2) 4.7 nM), and the PDE5 selective drugs sildenafil[184] (**51**, IC_{50} (PDE5) ~ 10 nM) and vardenafil[185] (**52**, IC_{50} (PDE5) < 1 nM).

Caffeine has not been cocrystallized with a PDE but structural insight can be obtained from IBMX-bound complex structures of PDE3, PDE4, PDE5, and PDE9 (Figure 4). The

https://doi.org/10.1021/acs.jmedchem.1c00261
J. Med. Chem. 2021, 64, 7156−7178

Scheme 8. PDE Inhibitors with Structural Relationship to Caffeine (Examples)



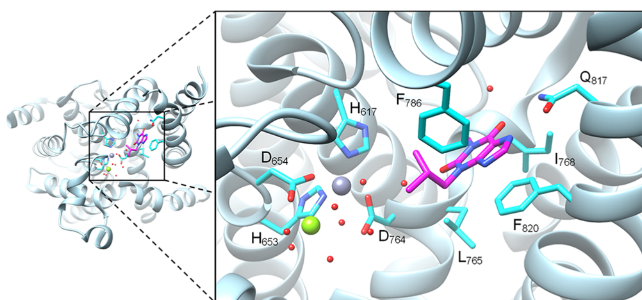


Figure 4. IBMX (**48**) bound to PDE5 (pdb 1RKP).

xanthine binds close to the catalytic site of PDEs which in PDE5 is composed of His617, His653, Asp654, and Asp764 that complex Mn^{2+} and Zn^{2+} as the catalytic center. IBMX binds to the same hydrophobic region of the pocket as the adenine or guanine motif of the PDE substrate and forms a π-stacking contact with Phe820. In addition, the carbonyl groups of IBMX participate in three H-bonds involving the side chain of Gln817 and two bound water molecules, which is a rare feature among the characterized xanthine binding sites since π-stacking and hydrophobic contacts usually dominate xanthine binding (see following chapters). Binding modes of IBMX in PDE3, PDE4, and PDE9 are very similar (not shown).

2.2.4. Caffeine as Ryanodine Receptor Modulator. Ryanodine receptors are exceptionally large (~500 kDa per subunit), tetrameric intracellular Ca^{2+} channels located at the endoplasmic or sarcoplasmic reticulum membrane and essential for intracellular Ca^{2+} mobilization. Three RyR isoforms have been identified of which the RyR1 is mainly found in skeletal muscle, RyR2 in myocardium, and RyR3 is ubiquitously expressed with high abundance in the CNS. Their activity is particularly important for muscle function in skeletal (RyR1) and cardiac (RyR2) muscles as well as for neuronal activity (RyR1, RyR2, and RyR3) by involving in excitation-contraction coupling and in neurotransmitter release.[97−99,186] RyR are sensitive to activation by cytosolic Ca^{2+} and thus mediate Ca^{2+}-induced Ca^{2+} release.[186] Additionally, several cellular signaling molecules are known to modulate RyR such as ATP and adenosine.[186] Early studies[95,96] have detected enhanced ryanodine binding to RyR and increased mean open time of RyR in the presence of caffeine, providing a mechanistic basis for previous observations that caffeine can induce muscle contraction[187] and intracellular Ca^{2+}-release from endoplasmic reticulum.[188,189] Therein, caffeine enhanced RyR Ca^{2+} affinity and revealed synergistic activity with adenine nucleotides, suggesting different binding sites.[190] Several studies have demonstrated that caffeine also activates RyR in neuronal cells.[191−193] These effects were, however, only demonstrated in vitro and observed at supraphysiological concentrations (1−20 mM) so far. Despite the hypothesis that sensitization of RyR by adenosine or other endogenous molecules increases potency of caffeine on RyR,[194] a physiological/pharmacological role of RyR modulation by caffeine remains to be confirmed.

Several cryo-EM structures have recently revealed the binding site of caffeine on RyR1 and RyR2 and elucidated activation and gating mechanisms.[97−99] Caffeine binds to four sites at the cytosolic side of tetrameric RyR (Figure 5) on the C-terminal domain which directly interacts with the pore helix. Both Ca^{2+} and ATP were discovered at different binding sites but in close proximity to caffeine. Binding of Ca^{2+} induces contraction of the central domain serving as a prerequisite for opening of the channel pore in a long-range allosteric mechanism. Caffeine binding leads to a constitutive contraction of the central domain which is further strengthened by ATP binding thereby explaining the synergistic activity of caffeine and adenine nucleotides. The caffeine binding site (one of four as example) is mostly defined by lipophilic amino acids (Phe3715, Trp4646, Ile4927, Tyr4945, Trp4646, Trp4942). Caffeine is sandwiched between Trp4646 and Ile4927 involving π-stacking with Trp4646 as a likely major contribution to binding. The caffeine binding site on RyR

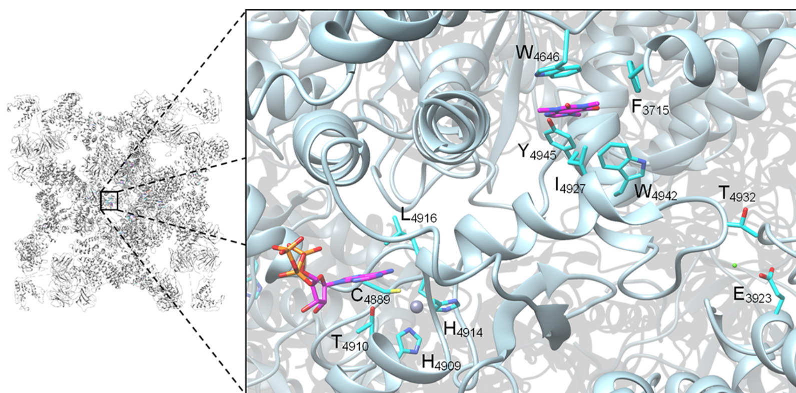


Figure 5. Binding of caffeine to ryanodine receptor (pdb 6JRS). One binding site is shown.

https://doi.org/10.1021/acs.jmedchem.1c00261
J. Med. Chem. 2021, 64, 7156−7178

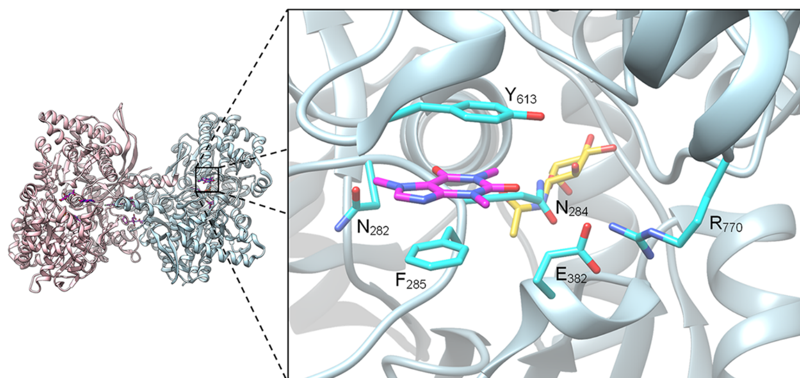


Figure 6. Glycogen phosphorylase with bound caffeine (pdb 1L5Q). Caffeine is shown in magenta, and *N*-acetyl-*β*-D-glucopyranosylamine is yellow.

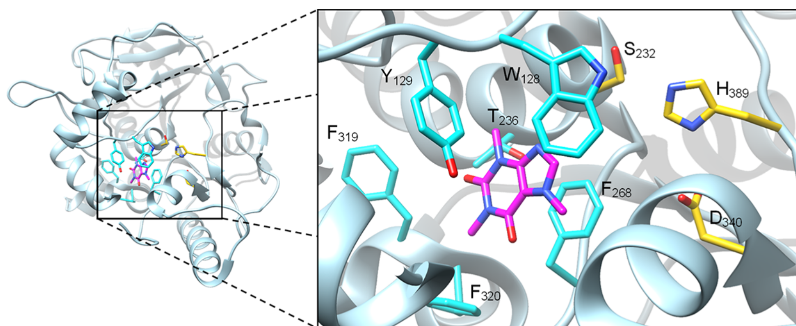


Figure 7. Notum in complex with caffeine (pdb 7TV4). Caffeine is shown in magenta, and the catalytic triad of Notum (Ser232, Asp340, and His389) is yellow.

provides several opportunities for structural extension and optimization.

For their crucial involvement in regulating intracellular Ca^{2+} homeostasis and excitation coupling, RyR arise as potential therapeutic targets in multiple diseases such as neurodegeneration, myopathies, malignant hyperthermia, and cardiovascular diseases.[195−198] For several such conditions, an association with mutations in RyR has been observed.[195−198] Apart from the RyR antagonist dantrolene,[199,200] tool compounds to pharmacologically validate RyR as therapeutic targets are lacking, however. The recent mechanistic findings on RyR modulation by caffeine and the pharmacological potential of RyR modulation may elicit stronger efforts to develop optimized RyR activators. Recent studies have reported high throughput compatible screening systems to characterize small molecule RyR modulators and discovered novel RyR ligands with higher potency than caffeine.[201−207] This progress provides a very valuable basis for medicinal chemistry toward potent RyR modulators which may also profit from structure-based design using the recently reported macromolecular RyR−caffeine complexes.

2.2.5. Caffeine as Ligand of Glycogen Phosphorylase. Glycogen phosphorylase catalyzes the rate-determining step of glycogenolysis and mediates the cleavage of glucose-1-phosphate from glycogen, thereby controlling the release of glucose from (hepatic) stores.[105,106] It is hence considered as a potential drug target to treat type 2 diabetes and related diseases. The enzyme has the special ability to switch between active and inactive states depending on an allosteric regulatory mechanism with several allosteric binding sites.[105,208] While AMP activates glycogen phosphorylase, glucose acts as an allosteric inhibitor by stabilizing the enzyme's inactive state as a negative feedback to block glycogen cleavage.[105,108,209,210] Caffeine was discovered as an allosteric modulator of glycogen phosphorylase and has synergistic activity with glucose.[105,108,210] It inhibited the enzyme alone and in the presence of the activator AMP.[210] An IC_{50} of 92 μM has been determined for caffeine alone, while addition of 0.2 mM AMP elevated the IC_{50} to 360 μM.[210] The combination of glucose and caffeine has been reported to increase potency by 2- to 3-fold in the presence and absence of AMP.[210] In accordance with the IC_{50}, SPR measurements have revealed an affinity of approximately 100 μM for caffeine binding to the purine binding site of apo glycogen phosphorylase.[105,108] The related xanthines **2** and **3** have slightly lower affinities. Medicinal chemistry efforts have yielded potent optimized ligands of both the catalytic site and the allosteric purine binding site but the compounds share little structural similarity with caffeine.[107,211]

Caffeine has been cocrystallized bound to the allosteric binding site of glycogen phosphorylase with a variety of synthetic inhibitors bound to the catalytic site. Caffeine binds between two aromatic residues (Phe285 and Tyr613) to engage π-stacking interactions (Figure 6). Both carbonyl oxygen atoms participate in water-mediated H-bonds to the protein backbone. Caffeine does not fully occupy its ample binding pocket which particularly offers additional space in the 1-, 3-, and 8-positions, and was found to accommodate also markedly larger molecules such as riboflavin.[105] The glucose binding site is located close to caffeine, rationalizing their synergy in modulating glycogen phosphorylase and a cross-talk between both binding sites.

2.2.6. Caffeine as Notum Inhibitor. Notum is a hydrolase enzyme found in humans and other metazoans, and was long considered as a secreted phospholipase cleaving, for example,

https://doi.org/10.1021/acs.jmedchem.1c00261
J. Med. Chem. 2021, 64, 7156−7178

heparan sulfate proteoglycans from cell surfaces, before it was identified as important feedback suppressor of Wnt signaling.[109] Therein, Notum also acts as a carboxylesterase, removing palmitoleate moieties from Ser187 on Wnt proteins.[109] Through this deacylation of Wnt proteins, Notum was found to inhibit Wnt signaling since the palmitoleate modification is required for formation of the Wnt-Frizzled complex and subsequent signal transduction.[109,212] Caffeine was discovered as an inhibitor of Notum with a binding affinity of 85 μM according to SPR measurements.[110] Moreover, caffeine blocked the activity of purified Notum with an IC_{50} value of 19 μM and restored Wnt signaling in a cellular reporter assay with an EC_{50} value of 46 μM.[110] Related xanthines were reported as markedly less active (theophylline, K_d 7 mM) or inactive (theobromine, paraxanthine).[110] Co-crystal structure elucidation of Notum in complex with caffeine revealed binding of caffeine at the active site close to the catalytic triad (Ser232, Asp340, and His389, Figure 7).[110] The alkaloid is bound in a very hydrophobic environment, which accommodates the lipophilic tail of the substrate palmitoleate, and engages a π-stacking interaction with Phe268. The free 8-position of caffeine points toward the catalytic triad. Considerable unoccupied space, and optimization potential seems to be available for development of caffeine derived high potency inhibitors of Notum. Since dysregulation of Wnt signaling has been implicated in various diseases including neurodegeneration and cancer, Notum inhibitors might hold great therapeutic potential. In the field of Alzheimer's Disease and other neurodegenerative pathologies, brain-penetrant Notum inhibitors need to be developed for which the discovery of caffeine binding to Notum and the associated structural information will be very valuable. In addition, Notum inhibition presents as another potential part of the complex puzzle of pharmacodynamic actions of caffeine, especially regarding its neuroprotective properties.

3. PHARMACOLOGICAL EFFECTS OF CAFFEINE

3.1. Pain and Headache. As an analgesic adjuvant, caffeine is found in many OTC formulations usually in combinations with acetaminophen, acetylsalicylic acid, or ibuprofen to treat headache and mild pain. Analgesic adjuvants do not have an analgesic effect on their own but enhance the effect of other analgesic agents.[213] It has been suggested that the analgesic adjuvant effect of caffeine may arise from its ability to promote absorption of analgesics by rapidly lowering gastric pH, thus affecting the pharmacokinetics of coadministered drugs.[24] Current evidence, however, rather points to adenosine-receptor-mediated activity involving the vasoconstrictor effects of A_{2A} blockade as a mechanistic basis of analgesic adjuvant properties.[27,34,214] A meta-analysis of 30 clinical trials with 10 000 patients with postpartum uterine cramping, episiotomy pain, postsurgical pain or headache receiving acetaminophen or acetylsalicylic acid with or without caffeine, found that an approximately 40% higher dose of the analgesic was needed without caffeine to achieve the same effect as the combined treatment.[215] In addition to the analgesic adjuvant use, a number of studies also point to moderate analgesic effects of caffeine monotherapy. A dose of 300 mg (oral) or 500 mg (intravenous) caffeine was shown to provide relief for patients with postdural puncture headache compared with placebo.[216,217] A moderate analgesic effect was also observed with 100 mg of caffeine in patients with tension-type headache or migraine.[218]

3.2. Apnea of Prematurity. Caffeine and theophylline are important drugs to treat neonatal apnea. Caffeine is preferred for its longer half-life and greater therapeutic index. A_1 and A_{2A} antagonism is considered as the main mode-of-action of xanthines in this indication.[219] A 2001 Cochrane Review of five clinical trials with a total of 192 preterm infants demonstrated that infants with apnea during the first 2−7 days benefited from treatment with a methylxanthine.[220] In a large study involving 2006 infants with birth weights between 500 and 1250 g, the therapeutic use of caffeine (20 mg/kg initial dose, 5 mg/kg maintenance dose) resulted in a lower risk of death or clinical disability and reduced the incidence of cerebral palsy and cognitive delay.[221]

3.3. Cardiovascular Health. Effects of caffeine on cardiovascular disease (CVD) have been studied for many years with contradictory results. Depending on the study, caffeine consumption was found to increase, have no effect, or decrease CVD risk. Linking, comparing, and evaluating these multiple studies is difficult, however, because the consumed amount of caffeine differed and was reported in different ways ranging between precise milligram amounts of caffeine to undefined cups of coffee. Another issue of several studies is the lack of placebo control which was in some cases defined as decaffeinated coffee, while other studies compared coffee drinkers and noncoffee drinkers. Potential cardiovascular effects reported for caffeine must hence be evaluated with care.

Nineteen observational cohort studies examined the association of caffeine consumption with CVD risk. The number of participants ranged between approximately 1000 and 100 000 with varying levels of coffee consumption. Caffeine was not taken in pure form but ingested through a beverage. Other ingredients of the drinks might hence be involved in the observed effect. Nonconsumers were analyzed as the control group. One study showed an increase in CVD risk as a function of age in adults who drank one or more cups of coffee compared to participants who consumed less than one cup. The correlation was weak, however, as also noted by the authors.[222] Four large cohort studies with participant numbers ranging from 3837 to 82 369 showed a statistically significant reduction of CVD risk in participants who consumed coffee or tea with up to a caffeine intake of >665 mg caffeine/day compared with nonusers or the lowest intake group.[223−226] Five other studies with participant numbers ranging from 6954 to 402 260 showed a protective effect for a caffeine consumption of up to 400 mg/day against CVD in a long follow-up period (8−28 years). No statistically significant change in CVD risk was observed in the group with high caffeine consumption between 400 and 600 mg/day compared with the group with lower consumption and the nonconsumers.[227−231] Nine further studies detected no statistically significant changes in CVD risk with caffeine consumption.[232−240] A 2013 meta-analysis pooled 36 studies with 1 279 804 participants and 36 352 cardiovascular disease cases and found a nonlinear association between coffee consumption and cardiovascular disease risk. The lowest risk of cardiovascular disease occurred with 3−5 cups per day, and no increasing risk was found with heavy coffee consumption.[241] Overall, moderate caffeine consumption with an intake of 100−400 mg per day might have some CVD protective effect, while evidence for beneficial effects of higher caffeine intake is weak.

3.4. Energy Drinks. Reports on potentially severe adverse cardiovascular effects of energy drink (ED) consumption draw

a different picture. In addition to coffee and tea, they have become a major source of caffeine intake and are particularly popular among adolescents and young adults also mixed with alcoholic beverages. The caffeine content of EDs varies between 32 and 134 mg of caffeine per 100 mL,[242] and additionally, EDs usually contain taurine, glucuronolactones, guarana, and vitamins.[243] Although some EDs do not exceed the caffeine content of coffee, caffeine uptake is enhanced since hot coffee is consumed slower and the caffeine is absorbed over a longer period of time.[244] Health risks associated with EDs are primarily attributed to the contained caffeine. Caffeine overdoses can cause arrhythmias, tachycardia, hypertension, palpitations, nausea, vomiting, stimulation of the nervous system, metabolic acidosis, convulsions, and in rare cases death.[245] Special care must also be taken when (excessive) caffeine intake is combined with physical exercise since it has been associated with diminished coronary artery flow reserve and enhanced myocardial oxygen demand.[246] A systematic review[247] has identified 17 cases of severe adverse events including two sudden deaths after ED consumption between 2001 and 2013. Even more alarming, 18 sudden deaths related to the consumption of EDs from various manufacturers were reported to the FDA between January 2004 and October 2012. The reported cases include healthy teenagers who died from cardiac arrhythmias and cardiac arrest but also have various confounding variables such as concomitant drug abuse, genetic predisposition, and heavy exercise.[248] Some recent case reports[249,250] provide further evidence for cardiomyopathy and acute kidney injury upon excessive ED consumption (up to 650 mg/d caffeine).

3.5. Neurodegenerative Diseases. Several lines of evidence point to a role of caffeine and coffee consumption in neurodegenerative diseases such as Alzheimer's Disease (AD), and Parkinson's Disease (PD). Epidemiological studies suggest especially a protective role of caffeine against these pathologies. In a transgenic mouse model of AD, caffeine administration (~1.5 mg/day) in young adulthood (4 months of age) led to improved memory performance and lower brain amyloid-β protein levels in later life of the mice (9 1/2 months). In addition, transgenic mice (18−19 months of age) that already showed cognitive impairment revealed restored memory function and brain amyloid-β levels after 2−3 months of treatment with caffeine in drinking water (0.3 mg/mL). The result was seen after 1−2 months of treatment with caffeine.[251,252] An inverse correlation between moderate caffeine/coffee consumption and risk of developing AD has also been discovered in several longitudinal studies in humans.[31,32,253,254] A case-control study[32] examining the caffeine intake of 54 patients with probable AD (73.9 ± 97.9 mg per day) versus 54 cognitively healthy patients (198.7 ± 135.7 mg per day) of matched age and sex over a 20-year period found a significant inverse association of caffeine consumption with AD. In a 10-year prospective cohort study[254] of 676 participants born between 1900 and 1920, men who drank coffee daily showed less cognitive decline than men who did not drink coffee. The least cognitive decline was seen with three cups of coffee per day. Similar results were obtained in a 21-year cardiovascular risk factors, aging and dementia study[31] with 1409 participants which also detected the lowest risk of developing AD with a daily coffee consumption of 3−5 cups.

A connection between caffeine/coffee consumption and lower PD risk is best documented. Multiple studies have shown that moderate consumption of caffeine can reduce the risk of developing PD without significant side effects on the cardiovascular system, bone status, or incidence of cancer.[30,52,81,255−264] One of the first studies in this context involving 8004 participants showed a reduction in the PD risk by 5-fold in participants who drank coffee (>421 mg caffeine per day) compared with noncoffee drinkers. In this study, the PD risk decreased with increasing coffee consumption.[258] A meta-analysis of 13 studies with a total of 901 764 participants also found an association between coffee intake and the risk of developing PD. With each 200 mg/day increase in caffeine consumption, the risk of PD decreased by 17%. The maximum protective effect was found for 3 cups of coffee per day,[30] and it was found that the effect of coffee on reduced risk of PD is largely due to the contained caffeine.[262]

The PD risk is not evenly distributed among the sexes and is lower in women than in men.[265] In postmenopausal women, an association was found between coffee consumption, hormone intake, and PD risk. Women with low caffeine intake (68 mg/day) had a lower risk of PD when they received hormone replacement therapy than without hormone intake. Only high caffeine intake (six or more cups of coffee, ≥688 mg/day caffeine) increased the PD risk in women with hormone replacement therapy compared with women who never drank coffee.[263]

Although some studies failed to detect beneficial effects of caffeine on AD risk,[266−268] protective effects of caffeine regarding PD risk are well documented, but the promising neuroprotective effects of caffeine require improved mechanistic understanding of the underlying molecular targets and signaling pathways.

Several mechanisms have been proposed to be involved in the beneficial effects including antagonism of adenosine receptors, regulation of cerebral blood flow, increase of oxygen consumption, and increase of cerebrospinal fluid production.[269−276] Recent studies indicate that interference with adenosinergic signaling, particularly A_{2A} blockade, is a key mechanism of the neuroprotective effects of caffeine.[28,29] Blockade of A_{2A} receptors, which are most abundant in the hippocampus, was found to prevent amyloid-β induced toxicity in the brain.[276,277] Accordingly, deletion of A_{2A} receptors in a mouse model of AD related tauopathy (THY-Tau22 mice) prevented memory deficits, impairment of hippocampal plasticity, and tau hyperphosphorylation. Similarly, A_{2A} antagonist treatment reduced tau hyperphosphorylation and ameliorated disease symptoms.[270]

Additionally, decreased cerebral perfusion resulting in hypoxia is a risk factor in AD[278] and two preliminary studies in men showed that administration of caffeine (100 mg) increased blood oxygen levels in certain regions of the brain.[271,273] Production and turnover of cerebrospinal fluid (CSF) were also found to be important in neurodegenerative diseases as high CSF turnover helps to transport toxic substances such as amyloid-β from the brain into the blood.[276] Caffeine was found to enhance the expression or activity of Na-K-ATPase, thereby providing another neuroprotective mechanism[279] since Na-K-ATPase plays a crucial role in the production and turnover of CSF.[275] Moreover, recently discovered molecular activities of caffeine such as modulation of the transcriptional regulator TLX, which is mainly found in neural stem cells as well as Notum inhibition resulting potentially in enhanced Wnt signaling, might

contribute to protective effects of caffeine against neurodegeneration.

4. SUMMARY AND PERSPECTIVE

The purine alkaloid caffeine is widely consumed in beverages for its stimulant properties which have been known for millennia. Caffeine was isolated and identified as the major stimulant constituent of coffee and tea two centuries ago, and its medicinal chemistry and pharmacology have been studied extensively for several decades. While its stimulant activity is well characterized and has been linked to a molecular mechanism in the 1980s when adenosine receptor blockade by caffeine was demonstrated, several other biological effects of caffeine remain at least partly elusive. Caffeine has been tested on multiple protein targets in vitro but part of the reported activities was observed at very high, supra-physiological concentrations. It is, therefore, likely that some reported effects of caffeine are nonspecific artifacts. However, a direct interaction of caffeine has been confirmed, for example, by cocrystal structures, also for proteins where the xanthine exhibits very low potency. Additionally, caffeine was found to synergize with endogenous molecules on some of its molecular targets suggesting potentially higher potency in vivo. It can hence be speculated that many biological effects of caffeine arise from the sum of several weak activities.

Medicinal chemistry research on caffeine has widely focused on its adenosine receptor antagonism which has yielded highly potent caffeine descendants as important tools to study the pharmacology of these receptors. The caffeine derivative istradefylline also acting as adenosine receptor antagonist has recently been introduced to the drug market. In addition, the phosphodiesterase inhibitory properties of caffeine have inspired extensive efforts to develop potent and selective PDE inhibitors, some of which have reached drug approval. For other confirmed protein targets, caffeine has not yet been extensively exploited as a lead compound by medicinal chemistry and further potential for drug discovery might rest in caffeine derived ligands for example of glycogen phosphorylase or the ryanodine receptor. Co-crystal structures and mechanistic insights in the modulation by caffeine are available for both proteins to guide medicinal chemistry efforts. The macromolecular data for both proteins provide insights into the caffeine binding sites and enable structure-based design of caffeine analogues with improved potency.

A particularly attractive pharmacological effect of caffeine was observed in the protective activity against neurodegenerative diseases that was found in several cohort studies. Despite some controversy regarding reduced AD risk with caffeine intake, the protective potential of caffeine consumption against PD is well documented. Several lines of evidence present adenosine receptor antagonism as a key mechanism underlying neuroprotective effects of caffeine, and accordingly, the caffeine-derived adenosine receptor antagonist istradefylline has recently been approved for PD treatment. Still, further molecular activities of caffeine are likely involved in the alkaloid's and its derivatives' neuroprotective properties as part of a complex picture of multiple caffeine actions in the CNS. Improved understanding of the neuroprotective mechanisms and their interplay might open new avenues for drug discovery, for example, in targeting the Wnt deacylase Notum or the ryanodine receptors.

Despite being one of the most widely consumed pharmacologically active molecules in the world, understanding of several aspects in the molecular and biological activities of caffeine is incomplete. While adenosine receptor antagonism has been linked with many of the attractive pharmacological effects of caffeine, additional molecular targets of caffeine are continuously discovered. Hence, several mechanisms likely contribute to pharmacological activities of caffeine, for example, in neurodegeneration. Given the lack of efficient therapeutics for severe health burdens as AD and PD, the macromolecular targets and mechanisms mediating the effects of caffeine appear as very promising avenues toward novel therapeutics especially in the field of neuroprotection. Although it has been in the focus of life-sciences for many decades, caffeine still presents as a very interesting molecule for pharmacological and medicinal chemistry research.

■ AUTHOR INFORMATION

Corresponding Author

Daniel Merk − *Institute of Pharmaceutical Chemistry, Goethe University Frankfurt, 60438 Frankfurt, Germany;* orcid.org/0000-0002-5359-8128; Email: merk@pharmchem.uni-frankfurt.de

Authors

Giuseppe Faudone − *Institute of Pharmaceutical Chemistry, Goethe University Frankfurt, 60438 Frankfurt, Germany*

Silvia Arifi − *Institute of Pharmaceutical Chemistry, Goethe University Frankfurt, 60438 Frankfurt, Germany*

Complete contact information is available at:
https://pubs.acs.org/10.1021/acs.jmedchem.1c00261

Author Contributions

[#]G.F. and S.A. contributed equally to this study

Notes

The authors declare no competing financial interest.

Biographies

Giuseppe Faudone obtained Bachelor and Master of Science degrees in Chemistry from Goethe University Frankfurt in 2015 and in 2017, respectively. He then joined the Institute of Pharmaceutical Chemistry at Goethe University Frankfurt as a Ph.D. student, where he currently focuses on the synthesis and characterization of nuclear receptor ligands.

Silvia Arifi studied pharmacy at Goethe University Frankfurt between 2015-2019 and received her license as a pharmacist in 2020. Currently, she is a Ph.D. student in Medicinal Chemistry at the Institute of Pharmaceutical Chemistry at the Goethe University focusing on the development and characterization of nuclear receptor ligands.

Daniel Merk graduated in Pharmaceutical Sciences and Pharmacy from LMU Munich in 2011 and obtained his Ph.D. in Pharmaceutical and Medicinal Chemistry from Goethe University Frankfurt in 2015. He was a Junior Group Leader in Pharmaceutical Chemistry at Goethe University Frankfurt and Postdoctoral ETH Fellowship Scholar at the Institute of Pharmaceutical Sciences of the Swiss Federal Institute of Technology (ETH) Zurich before he finished his Habilitation in Pharmaceutical Chemistry at Goethe University Frankfurt in 2019. Since then, he has been Independent Group Leader in Pharmaceutical Chemistry at Goethe University Frankfurt focusing on the Medicinal Chemistry and Pharmacology of nuclear receptors and their ligands. Recently, Daniel Merk joined LMU Munich as Chair of Pharmaceutical and Medicinal Chemistry.

https://doi.org/10.1021/acs.jmedchem.1c00261
J. Med. Chem. 2021, 64, 7156−7178

■ ACKNOWLEDGMENTS

Molecular graphics and analyses performed with UCSF Chimera, developed by the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco, with support from NIH P41-GM103311.

■ ABBREVIATIONS

AD, Alzheimer's Disease; ADOR, adenosine receptor; CNS, central nervous system; CSF, cerebrospinal fluid; CREBP, cyclic AMP response element binding protein; CVD, cardiovascular disease; CYP, cytochrome P450; ED, energy drink; $GABA_A$, γ-aminobutyric acid receptor A; GPCR, G-protein coupled receptor; hERG, human Ether-a-go-go-Related Gene; NAT2, N-acetyltransferase 2; NSAID, nonsteroidal anti-inflammatory drug; PD, Parkinson's Disease; PDE, phosphodiesterase; PI3K, phosphatidylinositol 3-kinase; PKA, protein kinase A; RyR, ryanodine receptor; TLX, tailless homologue receptor

■ REFERENCES

(1) Nehlig, A. Interindividual Differences in Caffeine Metabolism and Factors Driving Caffeine Consumption. *Pharmacol. Rev.* **2018**, *70* (2), 384−411.
(2) Evan, J. C.. *Tea in China: The History of China's National Drink*; Praeger Publishers Inc, 1992.
(3) *Chocolate in Health and Nutrition*; Watson, R. R., Preedy, V. R., Zibadi, S., Eds.; Humana Press, 2012.
(4) Weinberg, B. A.; Bealer, B. K.. *The World of Caffeine: The Science and Culture of the World's Most Popular Drug*; Routledge, 2002.
(5) Friedlieb Ferdinand, Runge. *Neueste Phytochemische Entdeckungen Zur Begründung Einer Wissenschaftlichen Phytochemie*; G. Reimer: Berlin, 1820.
(6) Mulder, G. J. Ueber Theïn Und Caffeïn. *J. Prakt. Chem.* **1838**, *15* (1), 280−284.
(7) Jobst, C. Thein Identisch Mit Caffein. *Ann. der Pharm.* **1838**, *25* (1), 63−66.
(8) Fischer, E.; Ach, L. Synthese Des Caffeïns. *Ber. Dtsch. Chem. Ges.* **1895**, *28* (3), 3135−3143.
(9) Fischer, E. Ueber Die Constitution Des Caffeïns, Xanthins, Hypoxanthins Und Verwandter Basen. *Ber. Dtsch. Chem. Ges.* **1897**, *30* (1), 549−559.
(10) Huang, R.; O'Donnell, A. J.; Barboline, J. J.; Barkman, T. J. Convergent Evolution of Caffeine in Plants by Co-Option of Exapted Ancestral Enzymes. *Proc. Natl. Acad. Sci. U. S. A.* **2016**, *113* (38), 10613−10618.
(11) Nathanson, J. A. Caffeine and Related Methylxanthines: Possible Naturally Occurring Pesticides. *Science* **1984**, *226* (4671), 184−187.
(12) Frischknecht, P. M.; Ulmer-Dufek, J.; Baumann, T. W. Purine Alkaloid Formation in Buds and Developing Leaflets of Coffea Arabica: Expression of an Optimal Defence Strategy? *Phytochemistry* **1986**, *25* (3), 613−616.
(13) López-González, L. M.; Sánchez-Oliver, A. J.; Mata, F.; Jodra, P.; Antonio, J.; Domínguez, R. Acute Caffeine Supplementation in Combat Sports: A Systematic Review. *J. Int. Soc. Sports Nutr.* **2018**, *15* (1), 60.
(14) Diel, P. Caffeine and Doping—What Have We Learned since 2004. *Nutrients* **2020**, *12* (8), 2167.
(15) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. Experimental and Computational Approaches to Estimate Solubility and Permeability in Drug Discovery and Development Settings. *Adv. Drug Delivery Rev.* **1997**, *23* (1−3), 3−25.
(16) Hansch, C.; Leo, A.; Hoekman, D. H. *Exploring QSAR.; ACS professional reference book*; American Chemical Society: Washington, DC, 1995.
(17) Wilkinson, S. C.; Maas, W. J. M.; Nielsen, J. B.; Greaves, L. C.; van de Sandt, J. J. M.; Williams, F. M. Interactions of Skin Thickness and Physicochemical Properties of Test Compounds in Percutaneous Penetration Studies. *Int. Arch. Occup. Environ. Health* **2006**, *79* (5), 405−413.
(18) Davies-Strickleton, H.; Cook, J.; Hannam, S.; Bennett, R.; Gibbs, A.; Edwards, D.; Ridden, C.; Ridden, J.; Cook, D. Assessment of the Nail Penetration of Antifungal Agents, with Different Physicochemical Properties. *PLoS One* **2020**, *15* (2), No. e0229414.
(19) Da Silva, R. S. Caffeine. In *Reproductive and Developmental Toxicology*; Gupta, R. C., Ed.; Elsevier Inc., 2011; pp 355−364.
(20) Comer, A. M.; Perry, C. M.; Figgitt, D. P. Caffeine Citrate: A Review of Its Use in Apnoea of Prematurity. *Paediatr. Drugs* **2001**, *3* (1), 61−79.
(21) Welsh, E. J.; Bara, A.; Barley, E.; Cates, C. J. Caffeine for Asthma. *Cochrane Database Syst. Rev.* **2010**, *2010* (1), CD001112.
(22) Alfaro, T. M.; Monteiro, R. A.; Cunha, R. A.; Cordeiro, C. R. Chronic Coffee Consumption and Respiratory Disease: A Systematic Review. *Clin. Respir. J.* **2018**, *12* (3), 1283−1294.
(23) Derry, C. J.; Derry, S.; Moore, R. A. Caffeine as an Analgesic Adjuvant for Acute Pain in Adults. *Cochrane Database Syst. Rev.* **2014**, *2017* (10), CD009281.
(24) Lipton, R. B.; Diener, H. C.; Robbins, M. S.; Garas, S. Y.; Patel, K. Caffeine in the Management of Patients with Headache. *J. Headache Pain* **2017**, *18* (1), 107.
(25) Weiser, T.; Weigmann, H. Effect of Caffeine on the Bioavailability and Pharmacokinetics of an Acetylsalicylic Acid-Paracetamol Combination: Results of a Phase I Study. *Adv. Ther.* **2019**, *36* (3), 597−607.
(26) Horrigan, L. A.; Kelly, J. P.; Connor, T. J. Immunomodulatory Effects of Caffeine: Friend or Foe? *Pharmacol. Ther.* **2006**, *111* (3), 877−892.
(27) Shapiro, R. E. Caffeine and Headaches. *Curr. Pain Headache Rep.* **2008**, *12* (4), 311−315.
(28) Silva, A. C.; Lemos, C.; Gonçalves, F. Q.; Pliássova, A. V.; Machado, N. J.; Silva, H. B.; Canas, P. M.; Cunha, R. A.; Lopes, J. P.; Agostinho, P. Blockade of Adenosine A2A Receptors Recovers Early Deficits of Memory and Plasticity in the Triple Transgenic Mouse Model of Alzheimer's Disease. *Neurobiol. Dis.* **2018**, *117*, 72−81.
(29) Cellai, L.; Carvalho, K.; Faivre, E.; Deleau, A.; Vieau, D.; Buée, L.; Blum, D.; Mériaux, C.; Gomez-Murcia, V. The Adenosinergic Signaling: A Complex but Promising Therapeutic Target for Alzheimer's Disease. *Front. Neurosci.* **2018**, *12*, 520.
(30) Qi, H.; Li, S. Dose-Response Meta-Analysis on Coffee, Tea and Caffeine Consumption with Risk of Parkinson's Disease. *Geriatr. Gerontol. Int.* **2014**, *14* (2), 430−439.
(31) Eskelinen, M. H.; Ngandu, T.; Tuomilehto, J.; Soininen, H.; Kivipelto, M. Midlife Coffee and Tea Drinking and the Risk of Late-Life Dementia: A Population-Based CAIDE Study. *J. Alzheimer's Dis.* **2009**, *16* (1), 85−91.
(32) Maia, L.; de Mendonca, A. Does Caffeine Intake Protect from Alzheimer's Disease? *Eur. J. Neurol.* **2002**, *9* (4), 377−382.
(33) Fong, L.; Hotson, A.; Powderly, J. D.; Sznol, M.; Heist, R. S.; Choueiri, T. K.; George, S.; Hughes, B. G. M.; Hellmann, M. D.; Shepard, D. R.; Rini, B. I.; Kummar, S.; Weise, A. M.; Riese, M. J.; Markman, B.; Emens, L. A.; Mahadevan, D.; Luke, J. J.; Laport, G.; Brody, J. D.; Hernandez-Aya, L.; Bonomi, P.; Goldman, J. W.; Berim, L.; Renouf, D. J.; Goodwin, R. A.; Munneke, B.; Ho, P. Y.; Hsieh, J.; McCaffery, I.; Kwei, L.; Willingham, S. B.; Miller, R. A. Adenosine 2A Receptor Blockade as an Immunotherapy for Treatment-Refractory Renal Cell Cancer. *Cancer Discovery* **2020**, *10* (1), 40−53.
(34) Jacobson, K. A.; Gao, Z. G.; Matricon, P.; Eddy, M. T.; Carlsson, J. Adenosine A2A Receptor Antagonists: From Caffeine to Selective Non-Xanthines. *Br. J. Pharmacol.* **2020**, in press. DOI: 10.1111/bph.15103.
(35) Blanchard, J.; Sawers, S. J. A. The Absolute Bioavailability of Caffeine in Man. *Eur. J. Clin. Pharmacol.* **1983**, *24* (1), 93−98.

(36) Arnaud, M. J. Metabolism of Caffeine and Other Components of Coffee. In *Caffeine, Coffee and Health*; Garattini, S., Ed.; Raven Press: New York, 1993.
(37) Benowitz, N. L. Clinical Pharmacology of Caffeine. *Annu. Rev. Med.* **1990**, *41*, 277−288.
(38) Doepker, C.; Lieberman, H. R.; Smith, A. P.; Peck, J. D.; El-Sohemy, A.; Welsh, B. T. Caffeine: Friend or Foe? *Annu. Rev. Food Sci. Technol.* **2016**, *7*, 117−137.
(39) Safranow, K.; Machoy, Z. Methylated Purines in Urinary Stones. *Clin. Chem.* **2005**, *51* (8), 1493−1498.
(40) Kalow, W.; Tang, B.-K. Use of Caffeine Metabolite Ratios to Explore CYP1A2 and Xanthine Oxidase Activities. *Clin. Pharmacol. Ther.* **1991**, *50* (5−1), 508−519.
(41) Whitfield, K.; Rambaldi, A.; Wetterslev, J.; Gluud, C. Pentoxifylline for Alcoholic Hepatitis. *Cochrane Database Syst. Rev.* **2009**, *2009* (4), CD007339.
(42) White, J. R.; Padowski, J. M.; Zhong, Y.; Chen, G.; Luo, S.; Lazarus, P.; Layton, M. E.; McPherson, S. Pharmacokinetic Analysis and Comparison of Caffeine Administered Rapidly or Slowly in Coffee Chilled or Hot versus Chilled Energy Drink in Healthy Young Adults. *Clin. Toxicol.* **2016**, *54* (4), 308−312.
(43) Gu, L.; Gonzalez, F. J.; Kalow, W.; Tang, B. K. Biotransformation of Caffeine, Paraxanthine, Theobromine and Theophylline by CDNA-Expressed Human CYP1A2 and CYP2E1. *Pharmacogenetics* **1992**, *2* (2), 73−77.
(44) Faber, M. S.; Jetter, A.; Fuhr, U. Assessment of CYP1A2 Activity in Clinical Practice: Why, How, and When? *Basic Clin. Pharmacol. Toxicol.* **2005**, *97* (3), 125−134.
(45) Cornelis, M. C.; Monda, K. L.; Yu, K.; Paynter, N.; Azzato, E. M.; Bennett, S. N.; Berndt, S. I.; Boerwinkle, E.; Chanock, S.; Chatterjee, N.; Couper, D.; Curhan, G.; Heiss, G.; Hu, F. B.; Hunter, D. J.; Jacobs, K.; Jensen, M. K.; Kraft, P.; Landi, M. T.; Nettleton, J. A.; Purdue, M. P.; Rajaraman, P.; Rimm, E. B.; Rose, L. M.; Rothman, N.; Silverman, D.; Stolzenberg-Solomon, R.; Subar, A.; Yeager, M.; Chasman, D. I.; van Dam, R. M.; Caporaso, N. E. Genome-Wide Meta-Analysis Identifies Regions on 7p21 (AHR) and 15q24 (CYP1A2) As Determinants of Habitual Caffeine Consumption. *PLoS Genet.* **2011**, *7* (4), No. e1002033.
(46) Aldridge, A.; Bailey, J.; Neims, A. H. The Disposition of Caffeine during and after Pregnancy. *Semin. Perinatol.* **1981**, *5* (4), 310−314.
(47) Kalow, W.; Tang, B. K. Caffeine as a Metabolic Probe: Exploration of the Enzyme-Inducing Effect of Cigarette Smoking. *Clin. Pharmacol. Ther.* **1991**, *49* (1), 44−48.
(48) Swanson, J. A.; Lee, J. W.; Hopp, J. W.; Berk, L. S. The Impact of Caffeine Use on Tobacco Cessation and Withdrawal. *Addict. Behav.* **1997**, *22* (1), 55−68.
(49) Rietveld, E. C.; Broekman, M. M. M.; Houben, J. J. G.; Eskes, T. K. A. B.; van Rossum, J. M. Rapid Onset of an Increase in Caffeine Residence Time in Young Women Due to Oral Contraceptive Steroids. *Eur. J. Clin. Pharmacol.* **1984**, *26* (3), 371−373.
(50) Bologa, M.; Tang, B.; Klein, J.; Tesoro, A.; Koren, G. Pregnancy-Induced Changes in Drug Metabolism in Epileptic Women. *J. Pharmacol. Exp. Ther.* **1991**, *257* (2), 735−740.
(51) Grosso, L. M.; Bracken, M. B. Caffeine Metabolism, Genetics, and Perinatal Outcomes: A Review of Exposure Assessment Considerations during Pregnancy. *Ann. Epidemiol.* **2005**, *15* (6), 460−466.
(52) Fredholm, B. B.; Bättig, K.; Holmén, J.; Nehlig, A.; Zvartau, E. E. Actions of Caffeine in the Brain with Special Reference to Factors That Contribute to Its Widespread Use. *Pharmacol. Rev.* **1999**, *51* (1), 83−133.
(53) Fenster, L.; Eskenazi, B.; Windham, G. C.; Swan, S. H. Caffeine Consumption during Pregnancy and Fetal Growth. *Am. J. Public Health* **1991**, *81* (4), 458−461.
(54) Wilcox, A.; Weinberg, C.; Baird, D. Caffeinated Beverages and Decreased Fertility. *Lancet* **1988**, *332* (8626−8627), 1453−1456.
(55) Cnattingius, S.; Signorello, L. B.; Annerén, G.; Clausson, B.; Ekbom, A.; Ljunger, E.; Blot, W. J.; McLaughlin, J. K.; Petersson, G.; Rane, A.; Granath, F. Caffeine Intake and the Risk of First-Trimester Spontaneous Abortion. *N. Engl. J. Med.* **2000**, *343* (25), 1839−1845.
(56) Wikoff, D.; Welsh, B. T.; Henderson, R.; Brorby, G. P.; Britt, J.; Myers, E.; Goldberger, J.; Lieberman, H. R.; O'Brien, C.; Peck, J.; Tenenbein, M.; Weaver, C.; Harvey, S.; Urban, J.; Doepker, C. Systematic Review of the Potential Adverse Effects of Caffeine Consumption in Healthy Adults, Pregnant Women, Adolescents, and Children. *Food Chem. Toxicol.* **2017**, *109*, 585−648.
(57) Hatch, E. E.; Bracken, M. B. Association of Delayed Conception with Caffeine Consumption. *Am. J. Epidemiol.* **1993**, *138* (12), 1082−1092.
(58) Lyngsø, J.; Ramlau-Hansen, C. H.; Bay, B.; Ingerslev, H. J.; Hulman, A.; Kesmodel, U. S. Association between Coffee or Caffeine Consumption and Fecundity and Fertility: A Systematic Review and Dose-Response Meta-Analysis. *Clinical Epidemiology* **2017**, *9*, 699.
(59) Sengpiel, V.; Elind, E.; Bacelis, J.; Nilsson, S.; Grove, J.; Myhre, R.; Haugen, M.; Meltzer, H. M.; Alexander, J.; Jacobsson, B.; Brantsæter, A. L. Maternal Caffeine Intake during Pregnancy Is Associated with Birth Weight but Not with Gestational Length: Results from a Large Prospective Observational Cohort Study. *BMC Med.* **2013**, *11* (1), 42.
(60) Weng, X.; Odouli, R.; Li, D. K. Maternal Caffeine Consumption during Pregnancy and the Risk of Miscarriage: A Prospective Cohort Study. *Am. J. Obstet. Gynecol.* **2008**, *198* (3), 279.e1−279.e8.
(61) CARE Study Group. Maternal Caffeine Intake during Pregnancy and Risk of Fetal Growth Restriction: A Large Prospective Observational Study. *BMJ.* **2008**, *337* (7682), 1334−1338.
(62) Chen, L. W.; Wu, Y.; Neelakantan, N.; Chong, M. F. F.; Pan, A.; van Dam, R. M. Maternal Caffeine Intake during Pregnancy Is Associated with Risk of Low Birth Weight: A Systematic Review and Dose-Response Meta-Analysis. *BMC Med.* **2014**, *12* (1), 174.
(63) Rhee, J.; Kim, R.; Kim, Y.; Tam, M.; Lai, Y.; Keum, N. N.; Oldenburg, C. E. Maternal Caffeine Consumption during Pregnancy and Risk of Low Birth Weight: A Dose-Response Meta-Analysis of Observational Studies. *PLoS One* **2015**, *10* (7), No. e0132334.
(64) Chen, L. W.; Fitzgerald, R.; Murrin, C. M.; Mehegan, J.; Kelleher, C. C.; Phillips, C. M. Associations of Maternal Caffeine Intake with Birth Outcomes: Results from the Lifeways Cross Generation Cohort Study. *Am. J. Clin. Nutr.* **2018**, *108* (6), 1301−1308.
(65) Mioranzza, S.; Nunes, F.; Marques, D. M.; Fioreze, G. T.; Rocha, A. S.; Botton, P. H. S.; Costa, M. S.; Porciúncula, L. O. Prenatal Caffeine Intake Differently Affects Synaptic Proteins during Fetal Brain Development. *Int. J. Dev. Neurosci.* **2014**, *36*, 45−52.
(66) Heckman, M. A.; Weil, J.; de Mejia, E. G. Caffeine (1, 3, 7-Trimethylxanthine) in Foods: A Comprehensive Review on Consumption, Functionality, Safety, and Regulatory Matters. *J. Food Sci.* **2010**, *75* (3), R77−R87.
(67) Law, V.; Knox, C.; Djoumbou, Y.; Jewison, T.; Guo, A. C.; Liu, Y.; Maciejewski, A.; Arndt, D.; Wilson, M.; Neveu, V.; Tang, A.; Gabriel, G.; Ly, C.; Adamjee, S.; Dame, Z. T.; Han, B.; Zhou, Y.; Wishart, D. S. DrugBank 4.0: Shedding New Light on Drug Metabolism. *Nucleic Acids Res.* **2014**, *42* (D1), D1091−D1097.
(68) Wishart, D. S.; Feunang, Y. D.; Guo, A. C.; Lo, E. J.; Marcu, A.; Grant, J. R.; Sajed, T.; Johnson, D.; Li, C.; Sayeeda, Z.; Assempour, N.; Iynkkaran, I.; Liu, Y.; MacIejewski, A.; Gale, N.; Wilson, A.; Chin, L.; Cummings, R.; Le, Di; Pon, A.; Knox, C.; Wilson, M. DrugBank 5.0: A Major Update to the DrugBank Database for 2018. *Nucleic Acids Res.* **2018**, *46* (D1), D1074−D1082.
(69) Juliano, L. M.; Griffiths, R. R. A Critical Review of Caffeine Withdrawal: Empirical Validation of Symptoms and Signs, Incidence, Severity, and Associated Features. *Psychopharmacology (Berl).* **2004**, *176* (1), 1−29.
(70) Cappelletti, S.; Daria, P.; Sani, G.; Aromatario, M. Caffeine: Cognitive and Physical Performance Enhancer or Psychoactive Drug? *Curr. Neuropharmacol.* **2015**, *13* (1), 71−88.

(71) Goasduff, T.; Dréano, Y.; Guillois, B.; Ménez, J. F.; Berthou, F. Induction of Liver and Kidney CYP1A1/1A2 by Caffeine in Rat. *Biochem. Pharmacol.* **1996**, *52* (12), 1915−1919.

(72) Guo, J.; Zhu, X.; Badawy, S.; Ihsan, A.; Liu, Z.; Xie, C.; Wang, X. Metabolism and Mechanism of Human Cytochrome P450 Enzyme 1A2. *Curr. Drug Metab.* **2021**, *22* (1), 40−49.

(73) Carrillo, J. A.; Benitez, J. Clinically Significant Pharmacokinetic Interactions between Dietary Caffeine and Medications. *Clin. Pharmacokinet.* **2000**, *39* (2), 127−153.

(74) Yartsev, A.; Peisah, C. Caffeine-Clozapine Interaction Associated with Severe Toxicity and Multiorgan System Failure: A Case Report. *BMC Psychiatry* **2021**, *21* (1), 192.

(75) Vainer, J. L.; Chouinard, G. Interaction between Caffeine and Clozapine. *J. Clin. Psychopharmacol.* **1994**, *14* (4), 284−285.

(76) Carrillo, J. A.; Herraiz, A. G.; Ramos, S. I.; Benítez, J. Effects of Caffeine Withdrawal from the Diet on the Metabolism of Clozapine in Schizophrenic Patients. *J. Clin. Psychopharmacol.* **1998**, *18* (4), 311−316.

(77) Sato, J.; Nakata, H.; Owada, E.; Kikuta, T.; Umetsu, M.; Ito, K. Influence of Usual Intake of Dietary Caffeine on Single-Dose Kinetics of Theophylline in Healthy Human Subjects. *Eur. J. Clin. Pharmacol.* **1993**, *44* (3), 295−298.

(78) Bento, A. P.; Gaulton, A.; Hersey, A.; Bellis, L. J.; Chambers, J.; Davies, M.; Krüger, F. A.; Light, Y.; Mak, L.; McGlinchey, S.; Nowotka, M.; Papadatos, G.; Santos, R.; Overington, J. P. The {ChEMBL} Bioactivity Database: An Update. *Nucleic Acids Res.* **2014**, *42* (D1), D1083−D1090.

(79) De Gubareff, T.; Sleator, W. Effects of Caffeine on Mammalian Atrial Muscle, and Its Interaction with Adexosine and Calcium. *J. Pharmacol. Exp. Ther.* **1965**, *148* (2), 202−214.

(80) Jacobson, K. A.; van Galen, P. J. M.; Williams, M. Adenosine Receptors: Pharmacology, Structure-Activity Relationships, and Therapeutic Potential. *J. Med. Chem.* **1992**, *35* (3), 407−422.

(81) Daly, J. W. Caffeine Analogs: Biomedical Impact. *Cell. Mol. Life Sci.* **2007**, *64* (16), 2153−2169.

(82) Choi, O. H.; Shamim, M. T.; Padgett, W. L.; Daly, J. W. Caffeine and Theophylline Analogues: Correlation of Behavioral Effects with Activity as Adenosine Receptor Antagonists and as Phosphodiesterase Inhibitors. *Life Sci.* **1988**, *43* (5), 387−398.

(83) Butcher, R.; Sutherland, W. Adenosine 3′,5′-Phosphate in Biological Materials. I. Purification and Properties of Cyclic 3′,5′-Nucleotide Phosphodiesterase and Use of This Enzyme to Characterize Adenosine 3′,5′-Phosphate in Human Urine. *J. Biol. Chem.* **1962**, *237*, 1244−1250.

(84) Beavo, J. A.; Reifsnyder, D. H. Primary Sequence of Cyclic Nucleotide Phosphodiesterase Isozymes and the Design of Selective Inhibitors. *Trends Pharmacol. Sci.* **1990**, *11* (4), 150−155.

(85) Shi, D.; Padgett, W. L.; Daly, J. W. Caffeine Analogs: Effects on Ryanodine-Sensitive Calcium-Release Channels and GABAA Receptors. *Cell. Mol. Neurobiol.* **2003**, *23* (3), 331−347.

(86) Marangos, P. J.; Paul, S. M.; Parma, A. M.; Goodwin, F. K.; Syapin, P.; Skolnick, P. Purinergic Inhibition of Diazepam Binding to Rat Brain (in Vitro). *Life Sci.* **1979**, *24* (9), 851−857.

(87) Nehlig, A.; Daval, J. L.; Debry, G. Caffeine and the Central Nervous System: Mechanisms of Action, Biochemical, Metabolic and Psychostimulant Effects. *Brain Res. Rev.* **1992**, *17* (2), 139−170.

(88) Fabiani, C.; Murray, A. P.; Corradi, J.; Antollini, S. S. A Novel Pharmacological Activity of Caffeine in the Cholinergic System. *Neuropharmacology* **2018**, *135*, 464−473.

(89) Okello, E. J.; Leylabi, R.; McDougall, G. J. Inhibition of Acetylcholinesterase by Green and White Tea and Their Simulated Intestinal Metabolites. *Food Funct.* **2012**, *3* (6), 651−661.

(90) Pohanka, M.; Dobes, P. Caffeine Inhibits Acetylcholinesterase, But Not Butyrylcholinesterase. *Int. J. Mol. Sci.* **2013**, *14* (5), 9873−9882.

(91) Karadsheh, N.; Kussie, P.; Linthicum, D. S. Inhibition of Acetylcholinesterase by Caffeine, Anabasine, Methyl Pyrrolidine and Their Derivatives. *Toxicol. Lett.* **1991**, *55* (3), 335−342.

(92) Wu, P.; Hu, Y. Small Molecules Targeting Phosphoinositide 3-Kinases. *MedChemComm* **2012**, *3* (11), 1337−1355.

(93) Saiki, S.; Sasazawa, Y.; Imamichi, Y.; Kawajiri, S.; Fujimaki, T.; Tanida, I.; Kobayashi, H.; Sato, F.; Sato, S.; Ishikawa, K. I.; Imoto, M.; Hattori, N. Caffeine Induces Apoptosis by Enhancement of Autophagy via PI3K/Akt/MTOR/P70S6K Inhibition. *Autophagy* **2011**, *7* (2), 176−187.

(94) Tariqul Islam, A. F. M.; Scavello, M.; Lotfi, P.; Daniel, D.; Haldeman, P.; Charest, P. G. Caffeine Inhibits PI3K and MTORC2 in Dictyostelium and Differentially Affects Multiple Other CAMP Chemoattractant Signaling Effectors. *Mol. Cell. Biochem.* **2019**, *457* (1−2), 157−168.

(95) McPhersonx, P. S.; Kim, Y. K.; Valdivia, H.; Knudson, C. M.; Takekura, H.; Franzini-Armstrong, C.; Coronadot, R.; Campbell, K. P. The Brain Ryanodine Receptor: A Caffeine-Sensitive Calcium Release Channel. *Neuron* **1991**, *7* (1), 17−25.

(96) Pessah, I. N.; Stambuk, R. A.; Casida, J. E. Ca2+-Activated Ryanodine Binding: Mechanisms of Sensitivity and Intensity Modulation by Mg2+, Caffeine, and Adenine Nucleotides. *Mol. Pharmacol.* **1987**, *31* (3), 232−238.

(97) des Georges, A.; Clarke, O. B.; Zalk, R.; Yuan, Q.; Condon, K. J.; Grassucci, R. A.; Hendrickson, W. A.; Marks, A. R.; Frank, J. Structural Basis for Gating and Activation of RyR1. *Cell* **2016**, *167* (1), 145−157.

(98) Chi, X.; Gong, D.; Ren, K.; Zhou, G.; Huang, G.; Lei, J.; Zhou, Q.; Yan, N. Molecular Basis for Allosteric Regulation of the Type 2 Ryanodine Receptor Channel Gating by Key Modulators. *Proc. Natl. Acad. Sci. U. S. A.* **2019**, *116* (51), 25575−25582.

(99) Gong, D.; Chi, X.; Wei, J.; Zhou, G.; Huang, G.; Zhang, L.; Wang, R.; Lei, J.; Chen, S. R. W.; Yan, N. Modulation of Cardiac Ryanodine Receptor 2 by Calmodulin. *Nature* **2019**, *572* (7769), 347−351.

(100) Warner, D. J.; Chen, H.; Cantin, L. D.; Kenna, J. G.; Stahl, S.; Walker, C. L.; Noeske, T. Mitigating the Inhibition of Human Bile Salt Export Pump by Drugs: Opportunities Provided by Physicochemical Property Modulation, in Silico Modeling, and Structural Modification. *Drug Metab. Dispos.* **2012**, *40* (12), 2332−2341.

(101) De Bruyn, T.; Van Westen, G. J. P.; Ijzerman, A. P.; Stieger, B.; De Witte, P.; Augustijns, P. F.; Annaert, P. P. Structure-Based Identification of Oatp1b1/3 Inhibitorss. *Mol. Pharmacol.* **2013**, *83* (6), 1257−1267.

(102) Sakatis, M. Z.; Reese, M. J.; Harrell, A. W.; Taylor, M. A.; Baines, I. A.; Chen, L.; Bloomer, J. C.; Yang, E. Y.; Ellens, H. M.; Ambroso, J. L.; Lovatt, C. A.; Ayrton, A. D.; Clarke, S. E. Preclinical Strategy to Reduce Clinical Hepatotoxicity Using in Vitro Bioactivation Data for > 200 Compounds. *Chem. Res. Toxicol.* **2012**, *25* (10), 2067−2082.

(103) Fernández, J. R.; Sweet, E. S.; Welsh, W. J.; Firestein, B. L. Identification of Small Molecule Compounds with Higher Binding Affinity to Guanine Deaminase (Cypin) than Guanine. *Bioorg. Med. Chem.* **2010**, *18* (18), 6748−6755.

(104) Cockerill, S. L.; Mitcheson, J. S. Direct Block of Human Ether-a-Go-Go-Related Gene Potassium Channels by Caffeine. *J. Pharmacol. Exp. Ther.* **2006**, *316* (2), 860−868.

(105) Ekstrom, J. L.; Pauly, T. A.; Carty, M. D.; Soeller, W. C.; Culp, J.; Danley, D. E.; Hoover, D. J.; Treadway, J. L.; Gibbs, E. M.; Fletterick, R. J.; Day, Y. S. N.; Myszka, D. G.; Rath, V. L. Structure-Activity Analysis of the Purine Binding Site of Human Liver Glycogen Phosphorylase. *Chem. Biol.* **2002**, *9* (8), 915−924.

(106) Thomson, S. A.; Banker, P.; Bickett, D. M.; Boucheron, J. A.; Carter, H. L.; Clancy, D. C.; Cooper, J. P.; Dickerson, S. H.; Garrido, D. M.; Nolte, R. T.; Peat, A. J.; Sheckler, L. R.; Sparks, S. M.; Tavares, F. X.; Wang, L.; Wang, T. Y.; Weiel, J. E. Anthranilimide Based Glycogen Phosphorylase Inhibitors for the Treatment of Type 2 Diabetes. Part 3: X-Ray Crystallographic Characterization, Core and Urea Optimization and in Vivo Efficacy. *Bioorg. Med. Chem. Lett.* **2009**, *19* (4), 1177−1182.

(107) Donnier-Maréchal, M.; Vidal, S. Glycogen Phosphorylase Inhibitors: A Patent Review (2013−2015). *Expert Opin. Ther. Pat.* **2016**, *26* (2), 199−212.
(108) Withers, S. G.; Sykes, B. D.; Madsen, N. B.; Kasvinsky, P. J. Identical Structural Changes Induced in Glycogen Phosphorylase by Two Nonexclusive Allosteric Inhibitors. *Biochemistry* **1979**, *18* (24), 5342−5348.
(109) Kakugawa, S.; Langton, P. F.; Zebisch, M.; Howell, S. A.; Chang, T. H.; Liu, Y.; Feizi, T.; Bineva, G.; O'Reilly, N.; Snijders, A. P.; Jones, E. Y.; Vincent, J. P. Notum Deacylates Wnt Proteins to Suppress Signalling Activity. *Nature* **2015**, *519* (7542), 187−192.
(110) Zhao, Y.; Ren, J.; Hillier, J.; Lu, W.; Jones, E. Y. Caffeine Inhibits Notum Activity by Binding at the Catalytic Pocket. *Commun. Biol.* **2020**, *3* (1), 555.
(111) Kopke, D. L.; Lima, S. C.; Alexandre, C.; Broadie, K. Notum Coordinates Synapse Development via Extracellular Regulation of Wingless Trans-Synaptic Signaling. *Development* **2017**, *144* (19), 3499−3510.
(112) Zhang, X.; Cheong, S. M.; Amado, N. G.; Reis, A. H.; MacDonald, B. T.; Zebisch, M.; Jones, E. Y.; Abreu, J. G.; He, X. Notum Is Required for Neural and Head Induction via Wnt Deacylation, Oxidation, and Inactivation. *Dev. Cell* **2015**, *32* (6), 719−730.
(113) Yoon, J. H.; Kim, D.; Kim, J.; Lee, H.; Ghim, J.; Kang, B. J.; Song, P.; Suh, P. G.; Ryu, S. H.; Lee, T. G. NOTUM Is Involved in the Progression of Colorectal Cancer. *Cancer Genomics Proteomics* **2018**, *15* (6), 485−497.
(114) De Robertis, M.; Arigoni, M.; Loiacono, L.; Riccardo, F.; Calogero, R. A.; Feodorova, Y.; Tashkova, D.; Belovejdov, V.; Sarafian, V.; Cavallo, F.; Signori, E. Novel Insights into Notum and Glypicans Regulation in Colorectal Cancer. *Oncotarget* **2015**, *6* (38), 41237−41257.
(115) Ukena, D.; Jacobson, K. A.; Padgett, W. L.; Ayala, C.; Shamim, M. T.; Kirk, K. L.; Olssont, R. O.; Daly, J. W. Species Differences in Structure-Activity Relationships of Adenosine Agonists and Xanthine Antagonists at Brain A1 Adenosine Receptors. *FEBS Lett.* **1986**, *209* (1), 122−128.
(116) Jacobson, K. A.; Ijzerman, A. P.; Müller, C. E. Medicinal Chemistry of P2 and Adenosine Receptors: Common Scaffolds Adapted for Multiple Targets. *Biochem. Pharmacol.* **2021**, *187*, 114311.
(117) Jacobson, K. A.; Müller, C. E. Medicinal Chemistry of Adenosine, P2Y and P2X Receptors. *Neuropharmacology* **2016**, *104*, 31−49.
(118) Müller, C. E.; Jacobson, K. A. Xanthines as Adenosine Receptor Antagonists. *Handbook of Experimental Pharmacology*; Springer: Berlin, Heidelberg, 2011; pp 151−199. DOI: 10.1007/978-3-642-13443-2_6.
(119) De Lera Ruiz, M.; Lim, Y. H.; Zheng, J. Adenosine A2A Receptor as a Drug Discovery Target. *J. Med. Chem.* **2014**, *57* (9), 3623−3650.
(120) Borea, P. A.; Varani, K.; Vincenzi, F.; Baraldi, P. G.; Tabrizi, M. A.; Merighi, S.; Gessi, S. The A3 Adenosine Receptor: History and Perspectives. *Pharmacol. Rev.* **2015**, *67* (1), 74−102.
(121) Zheng, J.; Zhang, X.; Zhen, X. Development of Adenosine A2A Receptor Antagonists for the Treatment of Parkinson's Disease: A Recent Update and Challenge. *ACS Chem. Neurosci.* **2019**, *10* (2), 783−791.
(122) Shimizu, H.; Daly, J. W.; Creveling, C. R. A Radioisotopic Method for Measuring the Formation of Adenosine 3′,5′-Cyclic Monophosphate in Incubated Slices of Brain. *J. Neurochem.* **1969**, *16* (12), 1609−1619.
(123) Londos, C.; Wolff, J. Two Distinct Adenosine-Sensitive Sites on Adenylate Cyclase. *Proc. Natl. Acad. Sci. U. S. A.* **1977**, *74* (12), 5482−5486.
(124) Calker, D.; Müller, M.; Hamprecht, B. Adenosine Regulates Via Two Different Types of Receptors, the Accumulation of Cyclic Amp in Cultured Brain Cells. *J. Neurochem.* **1979**, *33* (5), 999−1005.
(125) Bertil, B.; Abbracchio, M. P.; Burnstock, G.; Daly, J. W.; Harden, K. T.; Jacobson, K. A.; Leff, P.; Williams, M. IV. Nomenclature and Classification of Purinoreceptors. *Pharmacol. Rev.* **1994**, *46* (2), 143−156.
(126) Daly, J. W.; Butts-Lamb, P.; Padgett, W. Subclasses of Adenosine Receptors in the Central Nervous System: Interaction with Caffeine and Related Methylxanthines. *Cell. Mol. Neurobiol.* **1983**, *3* (1), 69−80.
(127) Zhou, Q. Y.; Li, L. I.; Olah, M. E.; Johnson, R. A.; Stiles, G. L.; Civelli, O. Molecular Cloning and Characterization of an Adenosine Receptor: The A3 Adenosine Receptor. *Proc. Natl. Acad. Sci. U. S. A.* **1992**, *89* (16), 7432−7436.
(128) Haskó, G.; Csóka, B.; Németh, Z. H.; Vizi, E. S.; Pacher, P. A2B Adenosine Receptors in Immunity and Inflammation. *Trends Immunol.* **2009**, *30* (6), 263−270.
(129) Daly, J. W.; Hide, I.; Müller, C. E.; Shamim, M. Caffeine Analogs: Structure Activity Relationship at Adenosine Receptors. *Pharmacology* **1991**, *42*, 309−321.
(130) Bruns, R. F.; Daly, J. W.; Snyder, S. H. Adenosine Receptors in Brain Membranes: Binding of N6-Cyclohexyl[3H]Adenosine and 1,3-Diethyl-8-[3H]Phenylxanthine. *Proc. Natl. Acad. Sci. U. S. A.* **1980**, *77* (9), 5547−5551.
(131) Bruns, R. F.; Daly, J. W.; Snyder, S. H. Adenosine Receptor Binding: Structure-Activity Analysis Generates Extremely Potent Xanthine Antagonists. *Proc. Natl. Acad. Sci. U. S. A.* **1983**, *80* (7), 2077−2080.
(132) Ukena, D.; Daly, J. W.; Kirk, K. L.; Jacobson, K. A. Functionalized Congeners of 1,3-Dipropyl-8-Phenylxanthine: Potent Antagonists for Adenosine Receptors That Modulate Membrane Adenylate Cyclase in Pheochromocytoma Cells, Platelets and Fat Cells. *Life Sci.* **1986**, *38*, 797−807.
(133) Jacobson, K. A.; Ukena, D.; Kirk, K. L.; Daly, J. W. [3H]Xanthine Amine Congener of 1,3-Dipropyl-8-Phenylxanthine: An Antagonist Radioligand for Adenosine Receptors. *Proc. Natl. Acad. Sci. U. S. A.* **1986**, *83* (11), 4089−4093.
(134) Bruns, R. F.; Fergus, J. H.; Badger, E. W.; Bristol, J. A.; Santay, L. A.; Hartman, J. D.; Hays, S. J.; Huang, C. C. Binding of the A1-Selective Adenosine Antagonist 8-Cyclopentyl-1,3-Dipropylxanthine to Rat Brain Membranes. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1987**, *335* (1), 59−63.
(135) Shimada, J.; Suzuki, F.; Nonaka, H.; Ishii, A. 8-Polycycloalkyl-1,3-Dipropylxanthines as Potent and Selective Antagonists for A1-Adenosine Receptors. *J. Med. Chem.* **1992**, *35* (5), 924−930.
(136) Belardinelli, L.; Shyrpock, J. C.; Zhang, Y.; Scammels, P. J.; Olsson, R.; Dennis, D.; Milner, P.; Pfister, J.; S. P, B. 1,3-Dipropyl-8-[2-(5,6-Epoxy)Norbornyl]Xanthine, a Potent, Specific and Selective A1 Adenosine Receptor Antagonist in the Guinea Pig Heart and Brain and in DDT1MF-2 Cells. *J. Pharmacol. Exp. Ther.* **1995**, *275*, 1167−1176.
(137) Daly, J. W.; Padgett, W.; Shamim, M. T.; Butts-Lamb, P.; Waters, J. 1,3-Dialkyl-8-(p-Sulfophenyl)Xanthines: Potent Water-Soluble Antagonists for A1 -and A2-Adenosine Receptors. *J. Med. Chem.* **1985**, *28* (4), 487−492.
(138) Hamilton, H. W.; Ortwine, D. F.; Worth, D. F.; Badger, E. W.; Bristol, J. A.; Bruns, R. F.; Haleen, S. J.; Steffen, R. P. Synthesis of Xanthines as Adenosine Antagonists, a Practical Quantitative Structure-Activity Relationship Application. *J. Med. Chem.* **1985**, *28* (8), 1071−1079.
(139) Shamim, M. T.; Ukena, D.; Padgett, W. L.; Hong, O.; Daly, J. W. 8-Aryl- and 8-Cycloalkyl-1,3-Dipropylxanthines: Further Potent and Selective Antagonists for A1-Adenosine Receptors. *J. Med. Chem.* **1988**, *31* (3), 613−617.
(140) Kiesman, W. F.; Zhao, J.; Conlon, P. R.; Dowling, J. E.; Petter, R. C.; Lutterodt, F.; Jin, X.; Smits, G.; Fure, M.; Jayaraj, A.; Kim, J.; Sullivan, G.; Linden, J. Potent and Orally Bioavailable 8-Bicyclo[2.2.2]Octylxanthines as Adenosine A1 Receptor Antagonists. *J. Med. Chem.* **2006**, *49* (24), 7119−7131.

(141) Ukena, D.; Shamim, M. T.; Padgett, W.; Daly, J. W. Analogs of Caffeine: Antagonists with Selectivity for A2 Adenosine Receptors. *Life Sci.* **1986**, *39*, 743−750.
(142) Seale, T. W.; Abla, K. A.; Shamim, M. T.; Carney, J. M.; Daly, J. W. 3,7-Dimethyl-1-Propargylxanthine: A Potent and Selective in Vivo Antagonist of Adenosine Analogs. *Life Sci.* **1988**, *43*, 1671−1684.
(143) Shamim, M. T.; Ukena, D.; Padgett, W. L.; Daly, J. W. Effects of 8-Phenyl and 8-Cycloalkyl Substituents on the Activity of Mono-, Di-, and Trisubstituted Alkylxanthines with Substitution at the 1-, 3-, and 7-Positions. *J. Med. Chem.* **1989**, *32* (6), 1231−1237.
(144) Shimada, J.; Suzuki, F.; Nonaka, H.; Ishii, A.; Ichikawa, S. (E)-1,3-Dialkyl-7-Methyl-8-(3,4,5-Trimethoxystyryl)Xanthines: Potent and Selective Adenosine A2 Antagonists. *J. Med. Chem.* **1992**, *35*, 2342−2345.
(145) Nonaka, H.; Ichimura, M.; Takeda, M.; Nonaka, Y.; Shimada, J.; Suzuki, F.; Yamaguchi, K.; Kase, H. KF17837 ((E)-8-(3,4-Dimethoxystyryl)- 1,3-Dipropyl-7-Methylxanthine), a Potent and Selective Adenosine A 2 Receptor Antagonist. *Eur. J. Pharmacol., Mol. Pharmacol. Sect.* **1994**, *267*, 335−341.
(146) Jacobson, K. A.; Gallo-Rodriguez, C.; Melman, N.; Fischer, B.; Maillard, M.; Van Bergen, A.; Van Galen, P. J. M.; Karton, Y. Structure-Activity Relationships of 8-Styrylxanthines as A2-Selective Adenosine Antagonists. *J. Med. Chem.* **1993**, *36* (10), 1333−1342.
(147) Jacobson, K. A.; Nikodijević, O.; Padgett, W. L.; Gallo-Rodriguez, C.; Maillard, M.; Daly, J. W. 8-(3-Chlorostyryl)Caffeine (CSC) Is a Selective A2-Adenosine Antagonist in Vitro and in Vivo. *FEBS Lett.* **1993**, *323* (1−2), 141−144.
(148) Chen, J. F.; Steyn, S.; Staal, R.; Petzer, J. P.; Xu, K.; Van Der Schyf, C. J.; Castagnoli, K.; Sonsalla, P. K.; Castagnoli, N.; Schwarzschild, M. A. 8-(3-Chlorostyryl) Caffeine May Attenuate MPTP Neurotoxicity through Dual Actions of Monoamine Oxidase Inhibition and A2A Receptor Antagonism. *J. Biol. Chem.* **2002**, *277* (39), 36040−36044.
(149) Dungo, R.; Deeks, E. D. Istradefylline: First Global Approval. *Drugs* **2013**, *73* (8), 875−882.
(150) FDA. Initial U.S. Approval for Nourianz. https://www.fda.gov/news-events/press-announcements/fda-approves-new-add-drug-treat-episodes-adults-parkinsons-disease, 2019.
(151) Kuder, K. J.; Załuski, M.; Schabikowski, J.; Latacz, G.; Olejarz-Maciej, A.; Jaśko, P.; Doroz-Płonka, A.; Brockmann, A.; Müller, C. E.; Kieć-Kononowicz, K. Novel, Dual Target-Directed Annelated Xanthine Derivatives Acting on Adenosine Receptors and Monoamine Oxidase B. *ChemMedChem* **2020**, *15* (9), 772−786.
(152) Załuski, M.; Schabikowski, J.; Schlenk, M.; Olejarz-Maciej, A.; Kubas, B.; Karcz, T.; Kuder, K.; Latacz, G.; Zygmunt, M.; Synak, D.; Hinz, S.; Müller, C. E.; Kieć-Kononowicz, K. Novel Multi-Target Directed Ligands Based on Annelated Xanthine Scaffold with Aromatic Substituents Acting on Adenosine Receptor and Monoamine Oxidase B. Synthesis, in Vitro and in Silico Studies. *Bioorg. Med. Chem.* **2019**, *27* (7), 1195−1210.
(153) Fredholm, B. B.; Persson, C. G. A. Xanthine Derivatives as Adenosine Receptor Antagonists. *Eur. J. Pharmacol.* **1982**, *81* (4), 673−676.
(154) Feoktistov, I.; Polosa, R.; Holgate, S. T.; Biaggioni, I. Adenosine A 2B Receptors: A Novel Therapeutic Target in Asthma? A Putative Role for Adenosine in Airway Inflammation. *Trends Pharmacol. Sci.* **1998**, *19*, 148−153.
(155) Kim, Y. C.; Ji, X. D.; Melman, N.; Linden, J.; Jacobson, K. A. Anilide Derivatives of an 8-Phenylxanthine Carboxylic Congener Are Highly Potent and Selective Antagonists at Human A(2B) Adenosine Receptors. *J. Med. Chem.* **2000**, *43* (6), 1165−1172.
(156) Hayallah, A. M.; Sandoval-Ramírez, J.; Reith, U.; Schobert, U.; Preiss, B.; Schumacher, B.; Daly, J. W.; Müller, C. E. 1,8-Disubstituted Xanthine Derivatives: Synthesis of Potent A2B-Selective Adenosine Receptor Antagonists. *J. Med. Chem.* **2002**, *45* (7), 1500−1510.
(157) Zablocki, J.; Kalla, R.; Perry, T.; Palle, V.; Varkhedkar, V.; Xiao, D.; Piscopio, A.; Maa, T.; Gimbel, A.; Hao, J.; Chu, N.; Leung, K.; Zeng, D. The Discovery of a Selective, High Affinity A2B Adenosine Receptor Antagonist for the Potential Treatment of Asthma. *Bioorg. Med. Chem. Lett.* **2005**, *15* (3), 609−612.
(158) Baraldi, P. G.; Tabrizi, M. A.; Preti, D.; Bovero, A.; Romagnoli, R.; Fruttarolo, F.; Zaid, N. A.; Moorman, A. R.; Varani, K.; Gessi, S.; Merighi, S.; Borea, P. A. Design, Synthesis, and Biological Evaluation of New 8-Heterocyclic Xanthine Derivatives as Highly Potent and Selective Human A2B Adenosine Receptor Antagonists. *J. Med. Chem.* **2004**, *47* (6), 1434−1447.
(159) Kalla, R. V.; Elzein, E.; Perry, T.; Li, X.; Palle, V.; Varkhedkar, V.; Gimbel, A.; Maa, T.; Zeng, D.; Zablocki, J. Novel 1,3-Disubstituted 8-(1-Benzyl-1H-Pyrazol-4-Yl) Xanthines: High Affinity and Selective A 2B Adenosine Receptor Antagonists. *J. Med. Chem.* **2006**, *49*, 3682−3692.
(160) Elzein, E.; Kalla, R. V.; Li, X.; Perry, T.; Gimbel, A.; Zeng, D.; Lustig, D.; Leung, K.; Zablocki, J. Discovery of a Novel A2B Adenosine Receptor Antagonist as a Clinical Candidate for Chronic Inflammatory Airway Diseases. *J. Med. Chem.* **2008**, *51* (7), 2267−2278.
(161) Kalla, R. V.; Elzein, E.; Perry, T.; Li, X.; Gimbel, A.; Yang, M.; Zeng, D.; Zablocki, J. Selective, High Affinity A2B Adenosine Receptor Antagonists: N-1 Monosubstituted 8-(Pyrazol-4-Yl)-Xanthines. *Bioorg. Med. Chem. Lett.* **2008**, *18* (4), 1397−1401.
(162) Baraldi, P. G.; Baraldi, S.; Saponaro, G.; Preti, D.; Romagnoli, R.; Piccagli, L.; Cavalli, A.; Recanatini, M.; Moorman, A. R.; Zaid, A. N.; Varani, K.; Borea, P. A.; Tabrizi, M. A. Novel 1,3-Dipropyl-8-(3-Benzimidazol-2-Yl-Methoxy-1-Methylpyrazol-5-Yl) Xanthines as Potent and Selective A2B Adenosine Receptor Antagonists. *J. Med. Chem.* **2012**, *55* (2), 797−811.
(163) Basu, S.; Barawkar, D. A.; Ramdas, V.; Patel, M.; Waman, Y.; Panmand, A.; Kumar, S.; Thorat, S.; Naykodi, M.; Goswami, A.; Reddy, B. S.; Prasad, V.; Chaturvedi, S.; Quraishi, A.; Menon, S.; Paliwal, S.; Kulkarni, A.; Karande, V.; Ghosh, I.; Mustafa, S.; De, S.; Jain, V.; Banerjee, E. R.; Rouduri, S. R.; Palle, V. P.; Chugh, A.; Mookhtiar, K. A. Design and Synthesis of Novel Xanthine Derivatives as Potent and Selective A2B Adenosine Receptor Antagonists for the Treatment of Chronic Inflammatory Airway Diseases. *Eur. J. Med. Chem.* **2017**, *134*, 218−229.
(164) Basu, S.; Barawkar, D. A.; Ramdas, V.; Waman, Y.; Patel, M.; Panmand, A.; Kumar, S.; Thorat, S.; Bonagiri, R.; Jadhav, D.; Mukhopadhyay, P.; Prasad, V.; Reddy, B. S.; Goswami, A.; Chaturvedi, S.; Menon, S.; Quraishi, A.; Ghosh, I.; Dusange, S.; Paliwal, S.; Kulkarni, A.; Karande, V.; Thakre, R.; Bedse, G.; Rouduri, S.; Gundu, J.; Palle, V. P.; Chugh, A.; Mookhtiar, K. A. A2B adenosine Receptor Antagonists: Design, Synthesis and Biological Evaluation of Novel Xanthine Derivatives. *Eur. J. Med. Chem.* **2017**, *127*, 986−996.
(165) Esteve, C.; Nueda, A.; Díaz, J. L.; Beleta, J.; Cárdenas, A.; Lozoya, E.; Cadavid, M. I.; Loza, M. I.; Ryder, H.; Vidal, B. New Pyrrolopyrimidin-6-Yl Benzenesulfonamides: Potent A2B Adenosine Receptor Antagonists. *Bioorg. Med. Chem. Lett.* **2006**, *16* (14), 3642−3645.
(166) Stefanachi, A.; Brea, J. M.; Cadavid, M. I.; Centeno, N. B.; Esteve, C.; Loza, M. I.; Martinez, A.; Nieto, R.; Raviña, E.; Sanz, F.; Segarra, V.; Sotelo, E.; Vidal, B.; Carotti, A. 1-, 3- and 8-Substituted-9-Deazaxanthines as Potent and Selective Antagonists at the Human A2B Adenosine Receptor. *Bioorg. Med. Chem.* **2008**, *16* (6), 2852−2869.
(167) Salvatore, C. A.; Jacobson, M. A.; Taylor, H. E.; Linden, J.; Johnson, R. G. Molecular Cloning and Characterization of the Human A3 Adenosine Receptor. *Proc. Natl. Acad. Sci. U. S. A.* **1993**, *90* (21), 10365−10369.
(168) Van Galen, P. J. M.; Van Bergen, A. H.; Gallo-Rodriguez, C.; Melman, N.; Olah, M. E.; Ijzerman, A. D. P.; Stiles, G. L.; Jacobson, K. A. A Binding Site Model and Structure-Activity Relationships for the Rat A3 Adenosine Receptor. *Mol. Pharmacol.* **1994**, *45* (6), 1101−1111.
(169) Kim, H. O.; Ji, X.-d.; Melman, N.; Olah, M. E.; Stiles, G. L.; Jacobson, K. A. Structure-Activity Relationships of 1,3-Dialkylxanthine Derivatives at Rat A3 Adenosine Receptors. *J. Med. Chem.* **1994**, *37* (20), 3373−3382.

(170) Priego, E. M.; Von Frijtag Drabbe Kuenzel, J.; Ijzerman, A. P.; Camarasa, M. J.; Pérez-Pérez, M. J. Pyrido[2,1-f]Purine-2,4-Dione Derivatives as a Novel Class of Highly Potent Human A3 Adenosine Receptor Antagonists. *J. Med. Chem.* **2002**, *45* (16), 3337−3344.

(171) Baraldi, P. G.; Preti, D.; Tabrizi, M. A.; Fruttarolo, F.; Romagnoli, R.; Zaid, N. A.; Moorman, A. R.; Merighi, S.; Varani, K.; Borea, P. A. New Pyrrolo[2,1-f]Purine-2,4-Dione and Imidazo[2,1-f]Purine-2,4-Dione Derivatives as Potent and Selective Human A3 Adenosine Receptor Antagonists. *J. Med. Chem.* **2005**, *48* (14), 4697−4701.

(172) Catarzi, D.; Colotta, V.; Varano, F.; Calabri, F. R.; Lenzi, O.; Filacchioni, G.; Trincavelli, L.; Martini, C.; Tralli, A.; Montopoli, C.; Moro, S. 2-Aryl-8-Chloro-1,2,4-Triazolo[1,5-a]Quinoxalin-4-Amines as Highly Potent A 1 and A 3 Adenosine Receptor Antagonists. *Bioorg. Med. Chem.* **2005**, *13* (3), 705−715.

(173) Ozola, V.; Thorand, M.; Diekmann, M.; Qurishi, R.; Schumacher, B.; Jacobson, K. A.; Müller, C. E. 2-Phenylimidazo-[2,1-i]Purin-5-Ones: Structure-Activity Relationships and Characterization of Potent and Selective Inverse Agonists at Human A3 Adenosine Receptors. *Bioorg. Med. Chem.* **2003**, *11* (3), 347−356.

(174) Xia, L.; Burger, W. A. C.; Van Veldhoven, J. P. D.; Kuiper, B. J.; Van Duijl, T. T.; Lenselink, E. B.; Paasman, E.; Heitman, L. H.; Ijzerman, A. P. Structure-Affinity Relationships and Structure-Kinetics Relationships of Pyrido[2,1-f]Purine-2,4-Dione Derivatives as Human Adenosine A3 Receptor Antagonists. *J. Med. Chem.* **2017**, *60* (17), 7555−7568.

(175) Yang, X.; Van Veldhoven, J. P. D.; Offringa, J.; Kuiper, B. J.; Lenselink, E. B.; Heitman, L. H.; Van Der Es, D.; Ijzerman, A. P. Development of Covalent Ligands for G Protein-Coupled Receptors: A Case for the Human Adenosine A3 Receptor. *J. Med. Chem.* **2019**, *62* (7), 3539−3552.

(176) Cheng, R. K. Y.; Segala, E.; Robertson, N.; Deflorian, F.; Doré, A. S.; Errey, J. C.; Fiez-Vandal, C.; Marshall, F. H.; Cooke, R. M. Structures of Human A1 and A2A Adenosine Receptors with Xanthines Reveal Determinants of Selectivity. *Structure* **2017**, *25* (8), 1275−1285.

(177) Doré, A. S.; Robertson, N.; Errey, J. C.; Ng, I.; Hollenstein, K.; Tehan, B.; Hurrell, E.; Bennett, K.; Congreve, M.; Magnani, F.; Tate, C. G.; Weir, M.; Marshall, F. H. Structure of the Adenosine A 2A Receptor in Complex with ZM241385 and the Xanthines XAC and Caffeine. *Structure* **2011**, *19* (9), 1283−1293.

(178) Francis, S. H.; Sekhar, K. R.; Ke, H.; Corbin, J. D. Inhibition of Cyclic Nucleotide Phosphodiesterases by Methylxanthines and Related Compounds. *Handb. Exp. Pharmacol.* **2011**, *200*, 93−133.

(179) Meskini, N.; Némoz, G.; Okyayuz-Baklouti, I.; Lagarde, M.; Prigent, A. F. Phosphodiesterase Inhibitory Profile of Some Related Xanthine Derivatives Pharmacologically Active on the Peripheral Microcirculation. *Biochem. Pharmacol.* **1994**, *47* (5), 781−788.

(180) Garst, J. E.; Kramer, G. L.; Wu, Y. J.; Wells, J. N. Inhibition of Separated Forms of Phosphodiesterases from Pig Coronary Arteries by Uracils and by 7-Substituted Derivatives of 1-Methyl-3-Isobutylxanthine. *J. Med. Chem.* **1976**, *19* (4), 499−503.

(181) Manallack, D. T.; Hughes, R. A.; Thompson, P. E. The next Generation of Phosphodiesterase Inhibitors: Structural Clues to Ligand and Substrate Selectivity of Phosphodiesterases. *J. Med. Chem.* **2005**, *48* (10), 3449−3462.

(182) Jansen, C.; Kooistra, A. J.; Kanev, G. K.; Leurs, R.; De Esch, I. J. P.; De Graaf, C. PDEStrIAn: A Phosphodiesterase Structure and Ligand Interaction Annotated Database As a Tool for Structure-Based Drug Design. *J. Med. Chem.* **2016**, *59* (15), 7029−7065.

(183) Boess, F. G.; Hendrix, M.; Van Der Staay, F. J.; Erb, C.; Schreiber, R.; Van Staveren, W.; De Vente, J.; Prickaerts, J.; Blokland, A.; Koenig, G. Inhibition of Phosphodiesterase 2 Increases Neuronal CGMP, Synaptic Plasticity and Memory Performance. *Neuropharmacology* **2004**, *47* (7), 1081−1092.

(184) Turko, I. V.; Ballard, S. A.; Francis, S. H.; Corbin, J. D. Inhibition of Cyclic GMP-Binding Cyclic GMP-Specific Phosphodiesterase (Type 5) by Sildenafil and Related Compounds. *Mol. Pharmacol.* **1999**, *56* (1), 124−130.

(185) Boyle, C. D.; Xu, R.; Asberom, T.; Chackalamannil, S.; Clader, J. W.; Greenlee, W. J.; Guzik, H.; Hu, Y.; Hu, Z.; Lankin, C. M.; Pissarnitski, D. A.; Stamford, A. W.; Wang, Y.; Skell, J.; Kurowski, S.; Vemulapalli, S.; Palamanda, J.; Chintala, M.; Wu, P.; Myers, J.; Wang, P. Optimization of Purine Based PDE1/PDE5 Inhibitors to a Potent and Selective PDE5 Inhibitor for the Treatment of Male ED. *Bioorg. Med. Chem. Lett.* **2005**, *15* (9), 2365−2369.

(186) Guerreiro, S.; Marien, M.; Michel, P. P. Methylxanthines and Ryanodine Receptor Channels. *Handbook of Experimental Pharmacology*; Springer: Berlin, Heidelberg, 2011; pp 135−150. DOI: 10.1007/978-3-642-13443-2_5.

(187) Conway, D.; Sakai, T. Caffeine Contracture. *Proc. Natl. Acad. Sci. U. S. A.* **1960**, *46* (6), 897−903.

(188) Endo, M. Calcium Release from the Sarcoplasmic Reticulum. *Physiol. Rev.* **1977**, *57* (1), 71−108.

(189) Fabiato, A. Calcium-Induced Release of Calcium from the Cardiac Sarcoplasmic Reticulum. *Am. J. Physiol. - Cell Physiol.* **1983**, *245* (1), C1−C14.

(190) McGarry, S. J.; Williams, A. J. Adenosine Discriminates between the Caffeine and Adenine Nucleotide Sites on the Sheep Cardiac Sarcoplasmic Reticulum Calcium-Release Channel. *J. Membr. Biol.* **1994**, *137* (2), 169−177.

(191) Kano, M.; Garaschuk, O.; Verkhratsky, A.; Konnerth, A. Ryanodine Receptor-mediated Intracellular Calcium Release in Rat Cerebellar Purkinje Neurones. *J. Physiol.* **1995**, *487* (1), 1−16.

(192) Patel, J. C.; Witkovsky, P.; Avshalumov, M. V.; Rice, M. E. Mobilization of Calcium from Intracellular Stores Facilitates Somatodendritic Dopamine Release. *J. Neurosci.* **2009**, *29* (20), 6568−6579.

(193) Sharma, G.; Vijayaraghavan, S. Modulation of Presynaptic Store Calcium Induces Release of Glutamate and Postsynaptic Firing. *Neuron* **2003**, *38* (6), 929−939.

(194) Magkos, F.; Kavouras, S. A. Caffeine Use in Sports, Pharmacokinetics in Man, and Cellular Mechanisms of Action. *Crit. Rev. Food Sci. Nutr.* **2005**, *45* (7−8), 535−562.

(195) Sun, L.; Wei, H. Ryanodine Receptors: A Potential Treatment Target in Various Neurodegenerative Disease. *Cell. Mol. Neurobiol.* **2020**, in press. DOI: 10.1007/s10571-020-00936-w.

(196) Shaaban, S.; Ramos-Platt, L.; Gilles, F. H.; Chan, W. M.; Andrews, C.; De Girolami, U.; Demer, J.; Engle, E. C. RYR1Mutations as a Cause of Ophthalmoplegia, Facial Weakness, and Malignant Hyperthermia. *JAMA Ophthalmol.* **2013**, *131* (12), 1532−1540.

(197) Lawal, T. A.; Todd, J. J.; Witherspoon, J. W.; Bönnemann, C. G.; Dowling, J. J.; Hamilton, S. L.; Meilleur, K. G.; Dirksen, R. T. Ryanodine Receptor 1-Related Disorders: An Historical Perspective and Proposal for a Unified Nomenclature. *Skeletal Muscle* **2020**, *10* (1), 32.

(198) Lawal, T. A.; Todd, J. J.; Meilleur, K. G. Ryanodine Receptor 1-Related Myopathies: Diagnostic and Therapeutic Approaches. *Neurotherapeutics* **2018**, *15* (4), 885−899.

(199) Kobayashi, S.; Bannister, M. L.; Gangopadhyay, J. P.; Hamada, T.; Parness, J.; Ikemoto, N. Dantrolene Stabilizes Domain Interactions within the Ryanodine Receptor. *J. Biol. Chem.* **2005**, *280* (8), 6580−6587.

(200) Diszházi, G.; Magyar, Z. É.; Mótyán, J. A.; Csernoch, L.; Jóna, I.; Nánási, P. P.; Almássy, J. Dantrolene Requires MG2+ and ATP to Inhibit the Ryanodine Receptor. *Mol. Pharmacol.* **2019**, *96* (3), 401−407.

(201) Murayama, T.; Kurebayashi, N. Assays for Modulators of Ryanodine Receptor (RyR)/Ca2+ Release Channel Activity for Drug Discovery for Skeletal Muscle and Heart Diseases. *Curr. Protoc. Pharmacol.* **2019**, *87* (1), No. e71.

(202) Murayama, T.; Kurebayashi, N.; Ishigami-Yuasa, M.; Mori, S.; Suzuki, Y.; Akima, R.; Ogawa, H.; Suzuki, J.; Kanemaru, K.; Oyamada, H.; Kiuchi, Y.; Iino, M.; Kagechika, H.; Sakurai, T. Efficient High-Throughput Screening by Endoplasmic Reticulum Ca 2+ Measurement to Identify Inhibitors of Ryanodine Receptor Ca 2+ -Release Channels. *Mol. Pharmacol.* **2018**, *94* (1), 722−730.

(203) Rebbeck, R. T.; Essawy, M. M.; Nitu, F. R.; Grant, B. D.; Gillispie, G. D.; Thomas, D. D.; Bers, D. M.; Cornea, R. L. High-Throughput Screens to Discover Small-Molecule Modulators of Ryanodine Receptor Calcium Release Channels. *SLAS Discovery* **2017**, *22* (2), 176−186.
(204) Rebbeck, R. T.; Singh, D. P.; Janicek, K. A.; Bers, D. M.; Thomas, D. D.; Launikonis, B. S.; Cornea, R. L. RyR1-Targeted Drug Discovery Pipeline Integrating FRET-Based High-Throughput Screening and Human Myofiber Dynamic Ca2+ Assays. *Sci. Rep.* **2020**, *10* (1), 1791.
(205) Mori, S.; Iinuma, H.; Manaka, N.; Ishigami-Yuasa, M.; Murayama, T.; Nishijima, Y.; Sakurai, A.; Arai, R.; Kurebayashi, N.; Sakurai, T.; Kagechika, H. Structural Development of a Type-1 Ryanodine Receptor (RyR1) Ca2+-Release Channel Inhibitor Guided by Endoplasmic Reticulum Ca2+ Assay. *Eur. J. Med. Chem.* **2019**, *179*, 837−848.
(206) Chen, J.; Xue, L.; Wei, R.; Liu, S.; Yin, C. C. The Insecticide Chlorantraniliprole Is a Weak Activator of Mammalian Skeletal Ryanodine Receptor/Ca 2+ Release Channel. *Biochem. Biophys. Res. Commun.* **2019**, *508* (2), 633−639.
(207) Diaz-Sylvester, P. L.; Porta, M.; Juettner, V. V.; Lv, Y.; Fleischer, S.; Copello, J. A. Eudistomin D and Penaresin Derivatives as Modulators of Ryanodine Receptor Channels and Sarcoplasmic Reticulum Ca2+ ATPase in Striated Muscle. *Mol. Pharmacol.* **2014**, *85* (4), 564−575.
(208) Oikonomakos, N. G.; Skamnaki, V. T.; Tsitsanou, K. E.; Gavalas, N. G.; Johnson, L. N. A New Allosteric Site in Glycogen Phosphorylase b as a Target for Drug Interactions. *Structure* **2000**, *8* (6), 575−584.
(209) Wang, J. H.; Shonka, M. L.; Graves, D. J. The Effect of Glucose on the Sedimentation and Catalytic Activity of Glycogen Phosphorylase. *Biochem. Biophys. Res. Commun.* **1965**, *18* (1), 131−135.
(210) Freeman, S.; Bartlett, J. B.; Convey, G.; Hardern, I.; Teague, J. L.; Loxham, S. J. G.; Allen, J. M.; Poucher, S. M.; Charles, A. D. Sensitivity of Glycogen Phosphorylase Isoforms to Indole Site Inhibitors Is Markedly Dependent on the Activation State of the Enzyme. *Br. J. Pharmacol.* **2006**, *149* (6), 775−785.
(211) Gaboriaud-Kolar, N.; Skaltsounis, A. L. Glycogen Phosphorylase Inhibitors: A Patent Review (2008−2012). *Expert Opin. Ther. Pat.* **2013**, *23* (8), 1017−1032.
(212) Janda, C. Y.; Waghray, D.; Levin, A. M.; Thomas, C.; Garcia, K. C. Structural Basis of Wnt Recognition by Frizzled. *Science* **2012**, *337* (6090), 59−64.
(213) Sawynok, J. Caffeine and Pain. *Pain* **2011**, *152* (4), 726−729.
(214) Sawynok, J. Adenosine Receptor Targets for Pain. *Neuroscience* **2016**, *338*, 1−18.
(215) Laska, E. M.; Sunshine, A.; Mueller, F.; Elvers, W. B.; Siegel, C.; Rubin, A. Caffeine as an Analgesic Adjuvant. *JAMA J. Am. Med. Assoc.* **1984**, *251* (13), 1711−1718.
(216) Camann, W. R.; Murray, R. S.; Mushlin, P. S.; Lambert, D. H. Effects of Oral Caffeine on Postdural Puncture Headache. A Double-Blind, Placebo-Controlled Trial. *Anesth. Analg.* **1990**, *70* (2), 181−184.
(217) Sechzer, P. H. Post-Spinal Anesthesia Headache Treated with Caffeine. Part II: Intracranial Vascular Distention, a Key Factor. *Curr. Ther. Res. - Clin. Exp.* **1979**, *26* (4), 440−448.
(218) Diener, H. C.; Pfaffenrath, V.; Pageler, L.; Peil, H.; Aicher, B. The Fixed Combination of Acetylsalicylic Acid, Paracetamol and Caffeine Is More Effective than Single Substances and Dual Combination for the Treatment of Headache: A Multicentre, Randomized, Double-Blind, Single-Dose, Placebo-Controlled Parallel Group St. *Cephalalgia* **2005**, *25* (10), 776−787.
(219) Eichenwald, E. C. Apnea of Prematurity. *Pediatrics* **2016**, *137* (1), No. e20153757.
(220) Henderson-Smart, D. J.; De Paoli, A. G.; Haughton, D. Methylxanthine Treatment for Apnoea in Preterm Infants. *Cochrane Database Syst. Rev.* **2010**, *2010* (12), CD000140.
(221) Schmidt, B.; Roberts, R. S.; Davis, P.; Doyle, L. W.; Barrington, K. J.; Ohlsson, A.; Solimano, A.; Tin, W. Caffeine Therapy for Apnea of Prematurity. *N. Engl. J. Med.* **2006**, *354* (20), 2112−2121.
(222) Lindsted, K. D.; Kuzma, J. W.; Anderson, J. L. Coffee Consumption and Cause-Specific Mortality Association with Age at Death and Compression of Mortality. *J. Clin. Epidemiol.* **1992**, *45* (7), 733−742.
(223) Bidel, S.; Hu, G.; Qiao, Q.; Jousilahti, P.; Antikainen, R.; Tuomilehto, J. Coffee Consumption and Risk of Total and Cardiovascular Mortality among Patients with Type 2 Diabetes. *Diabetologia* **2006**, *49* (11), 2618−2626.
(224) Kokubo, Y.; Iso, H.; Saito, I.; Yamagishi, K.; Yatsuya, H.; Ishihara, J.; Inoue, M.; Tsugane, S. The Impact of Green Tea and Coffee Consumption on the Reduced Risk of Stroke Incidence in Japanese Population: The Japan Public Health Center-Based Study Cohort. *Stroke* **2013**, *44* (5), 1369−1374.
(225) Kuriyama, S.; Shimazu, T.; Ohmori, K.; Kikuchi, N.; Nakaya, N.; Nishino, Y.; Tsubono, Y.; Tsuji, I. Green Tea Consumption and Mortality Due to Cardiovascular Disease, Cancer, and All Causes in Japan: The Ohsaki Study. *J. Am. Med. Assoc.* **2006**, *296* (10), 1255−1265.
(226) Suzuki, E.; Yorifuji, T.; Takao, S.; Komatsu, H.; Sugiyama, M.; Ohta, T.; Ishikawa-Takata, K.; Doi, H. Green Tea Consumption and Mortality among Japanese Elderly People: The Prospective Shizuoka Elderly Cohort. *Ann. Epidemiol.* **2009**, *19* (10), 732−739.
(227) Ding, M.; Satija, A.; Bhupathiraju, S. N.; Hu, Y.; Sun, Q.; Han, J.; Lopez-Garcia, E.; Willett, W.; Van Dam, R. M.; Hu, F. B. Association of Coffee Consumption with Total and Cause-Specific Mortality in 3 Large Prospective Cohorts. *Circulation* **2015**, *132* (24), 2305−2315.
(228) Freedman, N. D.; Park, Y.; Abnet, C. C.; Hollenbeck, A. R.; Sinha, R. Association of Coffee Drinking with Total and Cause-Specific Mortality. *N. Engl. J. Med.* **2012**, *366* (20), 1891−1904.
(229) Greenberg, J. A.; Dunbar, C. C.; Schnoll, R.; Kokolis, R.; Kokolis, S.; Kassotis, J. Caffeinated Beverage Intake and the Risk of Heart Disease Mortality in the Elderly: A Prospective Analysis. *Am. J. Clin. Nutr.* **2007**, *85* (2), 392−398.
(230) Mineharu, Y.; Koizumi, A.; Wada, Y.; Iso, H.; Watanabe, Y.; Date, C.; Yamamoto, A.; Kikuchi, S.; Inaba, Y.; Toyoshima, H.; Kondo, T.; Tamakoshi, A. Coffee, Green Tea, Black Tea and Oolong Tea Consumption and Risk of Mortality from Cardiovascular Disease in Japanese Men and Women. *J. Epidemiol. Community Health* **2011**, *65* (3), 230−240.
(231) Sugiyama, K.; Kuriyama, S.; Akhter, M.; Kakizaki, M.; Nakaya, N.; Ohmori-Matsuda, K.; Shimazu, T.; Nagai, M.; Sugawara, Y.; Hozawa, A.; Fukao, A.; Tsuji, I. Coffee Consumption and Mortality Due to All Causes, Cardiovascular Disease, and Cancer in Japanese Women. *J. Nutr.* **2010**, *140* (5), 1007−1013.
(232) Andersen, L. F.; Jacobs, D. R.; Carlsen, M. H.; Blomhoff, R. Consumption of Coffee Is Associated with Reduced Risk of Death Attributed to Inflammatory and Cardiovascular Diseases in the Iowa Women's Health Study. *Am. J. Clin. Nutr.* **2006**, *83* (5), 1039−1046.
(233) Happonen, P.; Läärä, E.; Hiltunen, L.; Luukinen, H. Coffee Consumption and Mortality in a 14-Year Follow-up of an Elderly Northern Finnish Population. *Br. J. Nutr.* **2008**, *99* (6), 1354−1361.
(234) Jacobsen, B. K.; Bjelke, E.; Kvale, G.; Heuch, I. Coffee Drinking, Mortality, and Cancer Incidence: Results from a Norwegian Prospective Study. *J. Natl. Cancer Inst.* **1986**, *76* (5), 823−831.
(235) Jazbec, A.; Simić, D.; Čorović, N.; Duraković, Z.; Pavlović, M. Impact of Coffee and Other Selected Factors on General Mortality and Mortality Due to Cardiovascular Disease in Croatia. *J. Heal. Popul. Nutr.* **2003**, *21* (4), 332−340.
(236) Lopez-Garcia, E.; Van Dam, R. M.; Li, T. Y.; Rodriguez-Artalejo, F.; Hu, F. B. The Relationship of Coffee Consumption with Mortality. *Ann. Intern. Med.* **2008**, *148* (12), 904−914.
(237) Martin, J. B.; Annegers, J. F.; Curb, J. D.; Heyden, S.; Howson, C.; Lee, E. S.; Lee, M. Mortality Patterns among Hypertensives by

Reported Level of Caffeine Consumption. *Prev. Med.* **1988**, *17* (3), 310−320.
(238) Paganini-Hill, A. Lifestyle Practices and Cardiovascular Disease Mortality in the Elderly: The Leisure World Cohort Study. *Cardiol. Res. Pract.* **2011**, *2011* (1), 983764.
(239) Silletta, M. G.; Marfisi, R.; Levantesi, G.; Boccanelli, A.; Chieffo, C.; Franzosi, M.; Geraci, E.; Maggioni, A. P.; Nicolosi, G.; Schweiger, C.; Tavazzi, L.; Tognoni, G.; Marchioli, R. Coffee Consumption and Risk of Cardiovascular Events after Acute Myocardial Infarction: Results from the GISSI (Gruppo Italiano per Lo Studio Della Sopravvivenza Nell'Infarto Miocardico)-Prevenzione Trial. *Circulation* **2007**, *116* (25), 2944−2951.
(240) Wilson, P. W. F.; Garrison, R. J.; Kannel, W. B.; McGee, D. L.; Castelli, W. P. Is Coffee Consumption a Contributor to Cardiovascular Disease? Insights from the Framingham Study. *Arch. Intern. Med.* **1989**, *149* (5), 1169−1172.
(241) Ding, M.; Bhupathiraju, S. N.; Satija, A.; Van Dam, R. M.; Hu, F. B. Long-Term Coffee Consumption and Risk of Cardiovascular Disease: A Systematic Review and a Dose-Response Meta-Analysis of Prospective Cohort Studies. *Circulation* **2014**, *129* (6), 643−659.
(242) Higgins, J. P.; Babu, K.; Deuster, P. A.; Shearer, J. Energy Drinks: A Contemporary Issues Paper. *Curr. Sports Med. Rep.* **2018**, *17* (2), 65−72.
(243) Higgins, J. P.; Tuttle, T. D.; Higgins, C. L. Energy Beverages: Content and Safety. *Mayo Clin. Proc.* **2010**, *85* (11), 1033−1041.
(244) Arria, A. M.; Caldeira, K. M.; Kasperski, S. J.; Vincent, K. B.; Griffiths, R. R.; O'Grady, K. E. Energy Drink Consumption and Increased Risk for Alcohol Dependence. *Alcohol.: Clin. Exp. Res.* **2011**, *35* (2), 365−375.
(245) Kerrigan, S.; Lindsey, T. Fatal Caffeine Overdose: Two Case Reports. *Forensic Sci. Int.* **2005**, *153* (1), 67−69.
(246) Higgins, J. P.; Babu, K. M. Caffeine Reduces Myocardial Blood Flow during Exercise. *Am. J. Med.* **2013**, *126* (8), 730.e1−730.e8.
(247) Goldfarb, M.; Tellier, C.; Thanassoulis, G. Review of Published Cases of Adverse Cardiovascular Events after Ingestion of Energy Drinks. *Am. J. Cardiol.* **2014**, *113* (1), 168−172.
(248) Higgins, J.; Yarlagadda, S.; Yang, B. Cardiovascular Complications of Energy Drinks. *Beverages* **2015**, *1* (2), 104−126.
(249) Al Yacoub, R.; Luczkiewicz, D.; Kerr, C. Acute Kidney Injury and Hepatitis Associated with Energy Drink Consumption: A Case Report. *J. Med. Case Rep.* **2020**, *14* (1), 23.
(250) Fisk, G.; Hammond-Haley, M.; D'Silva, A. Energy Drink-Induced Cardiomyopathy. *BMJ. Case Rep.* **2021**, *14* (4), No. e239370.
(251) Arendash, G. W.; Schleif, W.; Rezai-Zadeh, K.; Jackson, E. K.; Zacharia, L. C.; Cracchiolo, J. R.; Shippy, D.; Tan, J. Caffeine Protects Alzheimer's Mice against Cognitive Impairment and Reduces Brain β-Amyloid Production. *Neuroscience* **2006**, *142* (4), 941−952.
(252) Arendash, G. W.; Mori, T.; Cao, C.; Mamcarz, M.; Runfeldt, M.; Dickson, A.; Rezai-Zadeh, K.; Tan, J.; Citron, B. A.; Lin, X.; Echeverria, V.; Potter, H. Caffeine Reverses Cognitive Impairment and Decreases Brain Amyloid-β Levels in Aged Alzheimer's Disease Mice. *J. Alzheimer's Dis.* **2009**, *17* (3), 661−680.
(253) Lindsay, J.; Sykes, E.; McDowell, I.; Verreault, R.; Laurin, D. More Than the Epidemiology of Alzheimer's Disease: Contributions of the Canadian Study of Health and Aging. *Can. J. Psychiatry* **2004**, *49* (2), 83−91.
(254) van Gelder, B. M.; Buijsse, B.; Tijhuis, M.; Kalmijn, S.; Giampaoli, S.; Nissinen, A.; Kromhout, D. Coffee Consumption Is Inversely Associated with Cognitive Decline in Elderly European Men: The FINE Study. *Eur. J. Clin. Nutr.* **2007**, *61* (2), 226−232.
(255) Higdon, J. V.; Frei, B. Coffee and Health: A Review of Recent Human Research. *Crit. Rev. Food Sci. Nutr.* **2006**, *46* (2), 101−123.
(256) Van Dam, R. M.; Willett, W. C.; Manson, J. A. E.; Hu, F. B. Coffee, Caffeine, and Risk of Type 2 Diabetes: A Prospective Cohort Study in Younger and Middle-Aged U.S. Women. *Diabetes Care* **2006**, *29* (2), 398−403.
(257) Cadden, I. S. H.; Partovi, N.; Yoshida, E. M. Review Article: Possible Beneficial Effects of Coffee on Liver Disease and Function. *Aliment. Pharmacol. Ther.* **2007**, *26*, 1.
(258) Ross, G. W.; Abbott, R. D.; Petrovitch, H.; Morens, D. M.; Grandinetti, A.; Tung, K. H.; Tanner, C. M.; Masaki, K. H.; Blanchette, P. L.; Curb, J. D.; Popper, J. S.; White, L. R. Association of Coffee and Caffeine Intake with the Risk of Parkinson Disease. *J. Am. Med. Assoc.* **2000**, *283* (20), 2674−2679.
(259) Hernán, M. A.; Takkouche, B.; Caamaño-Isorna, F.; Gestal-Otero, J. J. A Meta-Analysis of Coffee Drinking, Cigarette Smoking, and the Risk of Parkinson's Disease. *Ann. Neurol.* **2002**, *52* (3), 276−284.
(260) Costa, J.; Lunet, N.; Santos, C.; Santos, J.; Vaz-Carneiro, A. Caffeine Exposure and the Risk of Parkinson's Disease: A Systematic Review and Meta-Analysis of Observational Studies. *J. Alzheimer's Dis.* **2010**, *20* (s1), S221−S238.
(261) Sääksjärvi, K.; Knekt, P.; Rissanen, H.; Laaksonen, M. A.; Reunanen, A.; Männistö, S. Prospective Study of Coffee Consumption and Risk of Parkinson's Disease. *Eur. J. Clin. Nutr.* **2008**, *62* (7), 908−915.
(262) Ascherio, A.; Zhang, S. M.; Hernán, M. A.; Kawachi, I.; Colditz, G. A.; Speizer, F. E.; Willett, W. C. Prospective Study of Caffeine Consumption and Risk of Parkinson's Disease in Men and Women. *Ann. Neurol.* **2001**, *50* (1), 56−63.
(263) Ascherio, A.; Chen, H.; Schwarzschild, M. A.; Zhang, S. M.; Colditz, G. A.; Speizer, F. E. Caffeine, Postmenopausal Estrogen, and Risk of Parkinson's Disease. *Neurology* **2003**, *60* (5), 790−795.
(264) Winkelmayer, W. C.; Stampfer, M. J.; Willett, W. C.; Curhan, G. C. Habitual Caffeine Intake and the Risk of Hypertension in Women. *J. Am. Med. Assoc.* **2005**, *294* (18), 2330−2335.
(265) Greene, N.; Lassen, C. F.; Rugbjerg, K.; Ritz, B. Reproductive Factors and Parkinson's Disease Risk in Danish Women. *Eur. J. Neurol.* **2014**, *21* (9), 1168−1177.
(266) Hameleers, P. A. H. M.; Van Boxtel, M. P. J.; Hogervorst, E.; Riedel, W. J.; Houx, P. J.; Buntinx, F.; Jolles, J. Habitual Caffeine Consumption and Its Relation to Memory, Attention, Planning Capacity and Psychomotor Performance across Multiple Age Groups. *Hum. Psychopharmacol.* **2000**, *15* (8), 573−581.
(267) Van Boxtel, M. P. J.; Schmitt, J. A. J.; Bosma, H.; Jolles, J. The Effects of Habitual Caffeine Use on Cognitive Change: A Longitudinal Perspective. *Pharmacol., Biochem. Behav.* **2003**, *75* (4), 921−927.
(268) Jarvis, M. J. Does Caffeine Intake Enhance Absolute Levels of Cognitive Performance? *Psychopharmacology (Berl).* **1993**, *110* (1−2), 45−52.
(269) Dall'Igna, O. P.; Fett, P.; Gomes, M. W.; Souza, D. O.; Cunha, R. A.; Lara, D. R. Caffeine and Adenosine A2a Receptor Antagonists Prevent β-Amyloid (25−35)-Induced Cognitive Deficits in Mice. *Exp. Neurol.* **2007**, *203* (1), 241−245.
(270) Laurent, C.; Burnouf, S.; Ferry, B.; Batalha, V. L.; Coelho, J. E.; Baqi, Y.; Malik, E.; Mariciniak, E.; Parrot, S.; Van Der Jeugd, A.; Faivre, E.; Flaten, V.; Ledent, C.; D'Hooge, R.; Sergeant, N.; Hamdane, M.; Humez, S.; Müller, C. E.; Lopes, L. V.; Buée, L.; Blum, D. A2A Adenosine Receptor Deletion Is Protective in a Mouse Model of Tauopathy. *Mol. Psychiatry* **2016**, *21* (1), 97−107.
(271) Koppelstaetter, F.; Poeppel, T. D.; Siedentopf, C. M.; Ischebeck, A.; Verius, M.; Haala, I.; Mottaghy, F. M.; Rhomberg, P.; Golaszewski, S.; Gotwald, T.; Lorenz, I. H.; Kolbitsch, C.; Felber, S.; Krause, B. J. Does Caffeine Modulate Verbal Working Memory Processes? An FMRI Study. *NeuroImage* **2008**, *39* (1), 492−499.
(272) Pelligrino, D. A.; Xu, H. L.; Vetri, F. Caffeine and the Control of Cerebral Hemodynamics. *J. Alzheimer's Dis.* **2010**, *20* (s1), S51−S62.
(273) Klaassen, E. B.; De Groot, R. H. M.; Evers, E. A. T.; Snel, J.; Veerman, E. C. I.; Ligtenberg, A. J. M.; Jolles, J.; Veltman, D. J. The Effect of Caffeine on Working Memory Load-Related Brain Activation in Middle-Aged Males. *Neuropharmacology* **2013**, *64*, 160−167.
(274) Haller, S.; Rodriguez, C.; Moser, D.; Toma, S.; Hofmeister, J.; Sinanaj, I.; Van De Ville, D.; Giannakopoulos, P.; Lovblad, K. O.

Acute Caffeine Administration Impact on Working Memory-Related Brain Activation and Functional Connectivity in the Elderly: A BOLD and Perfusion MRI Study. *Neuroscience* **2013**, *250*, 364−371.

(275) Han, M. E.; Kim, H. J.; Lee, Y. S.; Kim, D. H.; Choi, J. T.; Pan, C. S.; Yoon, S.; Baek, S. Y.; Kim, B. S.; Kim, J. B.; Oh, S. O. Regulation of Cerebrospinal Fluid Production by Caffeine Consumption. *BMC Neurosci.* **2009**, *10*, 110.

(276) Wostyn, P.; Van Dam, D.; Audenaert, K.; De Deyn, P. P. Increased Cerebrospinal Fluid Production as a Possible Mechanism Underlying Caffeine's Protective Effect against Alzheimer's Disease. *Int. J. Alzheimer's Dis.* **2011**, *2011*, 617420.

(277) Dall'lgna, O. P.; Porciúncula, L. O.; Souza, D. O.; Cunha, R. A.; Lara, D. R. Neuroprotection by Caffeine and Adenosine A 2A Receptor Blockade of β-Amyloid Neurotoxicity. *Br. J. Pharmacol.* **2003**, *138* (7), 1207−1209.

(278) Sun, X.; He, G.; Qing, H.; Zhou, W.; Dobie, F.; Cai, F.; Staufenbiel, M.; Huang, L. E.; Song, W. Hypoxia Facilitates Alzheimer's Disease Pathogenesis by up-Regulating BACE1 Gene Expression. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103* (49), 18727−18732.

(279) Zhang, X.; Wu, M.; Lu, F.; Luo, N.; He, Z. P.; Yang, H. Involvement of A7 NAChR Signaling Cascade in Epigallocatechin Gallate Suppression of β-Amyloid-Induced Apoptotic Cortical Neuronal Insults. *Mol. Neurobiol.* **2014**, *49* (1), 66−77.

https://doi.org/10.1021/acs.jmedchem.1c00261
J. Med. Chem. 2021, 64, 7156−7178