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Original article

Novel structural hybrids of pyrazolobenzothiazines with benzimidazoles as cholinesterase inhibitors

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ABSTRACT

Two series of novel pyrazolobenzothiazine-based hybrid compounds were efficiently synthesized starting from saccharin sodium salt. Pyrazolo[4,3-*c*][1,2]benzothiazine scaffolds were *N*-arylated by using *p*-fluorobenzaldehyde, followed by the incorporation of a benzimidazole or similar ring systems by treatment with arylenediamines. These phenylene-connected hybrid compounds were investigated as potential inhibitors of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). Compounds **12d** and **12k** were the most potent AChE inhibitors with IC_{50} values of 11 and 13 nM, respectively, while **6j** (IC_{50} = 17 nM) proved to be the most active inhibitor against BuChE with remarkable selectivity for BuChE over AChE. Molecular docking studies were also performed on human AChE and BuChE to suggest possible binding modes in which the inhibitor's extended structure is accommodated along the active site gorge of both enzymes.

1. Introduction

Cholinergic neurons of the basal forebrain play an important role in cognitive and behavioural functions, with extensive cortical projections that modulate other neurotransmitters [1]. These cholinergic systems are known to be widely compromised in Alzheimer's disease (AD). In particular, AD is characterized by a forebrain cholinergic neuronal loss, a progressive cognitive decline and neuropsychiatric disturbances in acetylcholine (ACh) [2]. The activity of ACh in the brain is terminated by the hydrolytic action of cholinesterases (ChEs). Inhibitors of these enzymes have hence been developed to augment the activity of surviving cholinergic neurons in patients with AD. All ChE inhibitors currently licensed for AD inhibit acetylcholinesterase (AChE) and, to a varying extent, butyrylcholinesterase (BuChE), a second ChE in the brain [3]. AChE and BuChE have numerous physiological functions depending on their localization and time of expression [4]. Therefore, both enzymes are likely to be involved in regulating ACh levels and represent legitimate therapeutic targets to ameliorate the cholinergic deficit. BuChE may also have a role in the aetiology and progression of AD beyond regulation of synaptic ACh levels [5]. The deficit of presynaptic markers is an indication of a neuronal degeneration which is the real cause of the profound depletion of ACh within the hippocampus, basal forebrain and cortical areas [6]. Since changes in cholinergic regulation are a contributing factor to the cognitive dysfunction observed in AD patients, the selective AChE inhibitor, donepezil, is widely utilized for symptomatic treatment of patients [7].

AChE is principally associated with neurons and axons, while BuChE is primarily expressed and secreted by glial cells within the brain [8]. Studies indicate that specific neurons use BuChE rather than AChE to cleave presynaptic ACh. Indeed, 10–15% of cholinergic neurons in the human hippocampus and amygdala express BuChE in their cell bodies and proximal dendrites, in lieu of AChE [9,10]. In

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the healthy human brain, AChE and BuChE are found in the ratio of 4:1. However, in the brains of AD patients, AChE activity can decline by up to 45% during disease progression, reflecting the disappearance of neurons and axons to which it is associated, while BuChE activity can be elevated by up to 2-fold [10], thereby altering this ratio considerably.

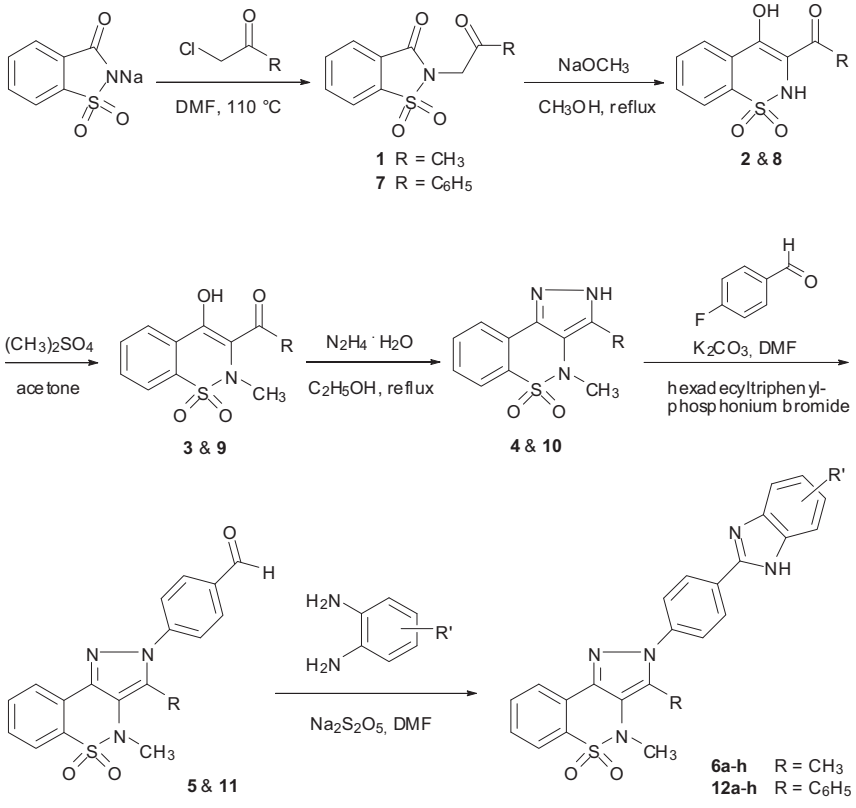
The benzothiazine ring systems have emerged as suitable heterocyclic templates for biologically active compounds. For example, 4*H*-3,1-benzothiazin-4-ones have been characterised as serine protease inhibitors [11] and as potent, dual-acting compounds targeting adenosine A_{2A} receptors and monoamine oxidase B [12]. Among the isomeric 4*H*-1,3-benzothiazin-4-ones, extremely effective antimycobacterial nitro derivatives have been explored [13]. 1,4-Benzothiazine derivatives are known to possess anticancer [14] and antibacterial [15] activities and act as activators of ATP-sensitive potassium channels [16]. Antimicrobial and anti-inflammatory properties have been reported for 1,2-benzothiazine 1,1-dioxides [17–19]. Among the tricyclic benzothiazine derivatives, pyrazolo[4,3-*c*][1,2]benzothiazine 5,5-dioxides possess anti-inflammatory [20] and antiviral [21] properties. Biological activity was demonstrated for similar fused benzothiazine templates, *e.g.* for pyrazolo[4,3-*c*][2,1]benzothiazine 4,4-dioxides acting as selective focal adhesion kinase inhibitors [22] and pyrazolo[4,3-*b*][1,4]benzothiazine 4,4-dioxides with antimalarial properties [23]. Thus, these molecules exhibit a wide range of biological activities and are considered to be privileged structures for medicinal chemistry. With respect to the versatile bioactivity profiles of pyrazolobenzothiazine pharmacophores, some of us explored 2,4-dihydro-3,4-dimethylpyrazolo[4,3-*c*][1,2]benzothiazine 5,5-dioxide derivatives as antioxidants and antimicrobial agents [24,25].

The benzimidazole ring system is another molecular template to generate biologically active compounds. Benzimidazole derivatives showed significant activities against a number of viruses including HIV [26,27], herpes (HSV-1 and HCMV) [28] and hepatitis C [29]. Various benzimidazoles also have a potential as antitumour [30,31] or antiparasitic agents [32] and as angiotensin II receptor antagonists [33]. Derivatives containing this pharmacophore are also used as ligands to transition metals, designed for biological system modelling [34].

On the basis of the biological activity profiles of the two pharmacophores *i.e.* pyrazolobenzothiazine and benzimidazole, we have hybridized these two ring systems into one unit. Our current study describes the discovery and structure–activity relationships of novel pyrazolobenzothiazine derivatives as inhibitors of cholinesterases. The active site of cholinesterases is located at the bottom of a deep and narrow gorge. Thus, the extended structure of the inhibitors designed in this study might be accommodated along the active site gorge, as it has been explored for several heterodimeric, linker connected inhibitors of cholinesterases [35–37].

2. Chemistry

We designed the synthetic route to two novel series of hybrids of benzimidazole-substituted pyrazolobenzothiazines as depicted in Scheme 1. The initial *N*-alkylation of the commercially available saccharin sodium salt with appropriate alkyl halides in DMF [25,38] was carried out under optimized conditions, *i.e. via* a thermal method, microwave irradiation and in an ultrasonic bath. Excellent yields of the product were obtained under microwave irradiation after a short reaction time. Then, the 1,2-benzothiazine nuclei (**2** and **8**) were prepared in an inert nitrogen atmosphere [39] *via* Gabriel–Colman-type ring expansion of the five-membered benzoisothiazole rings of compound **1** and **7** to the six-membered thiazine ring, including the ring cleavage and ring closure steps. The subsequent *N*-methylation of compounds **2** and **8** with dimethyl sulphate in acetone was performed under ultrasonic



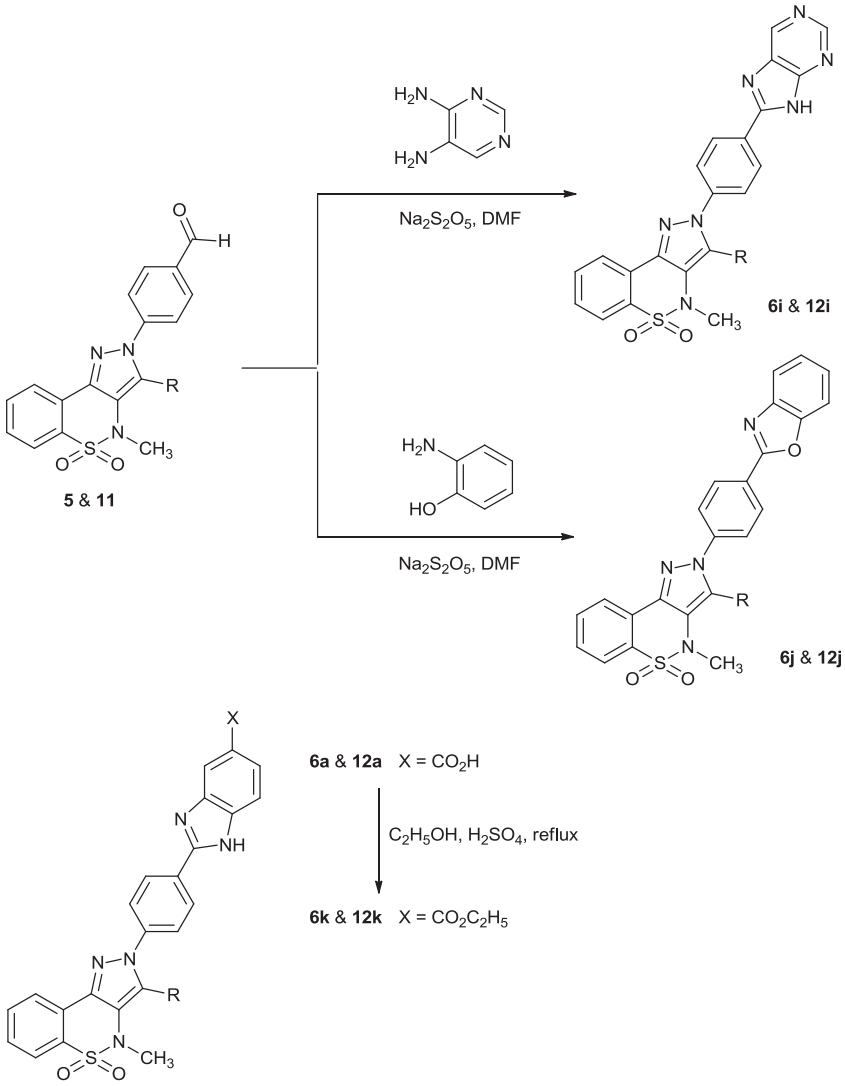
Scheme 1. Synthesis of pyrazolo[4,3-*c*][1,2]benzothiazine 5,5-dioxides (**6a–h** and **12a–h**).

conditions for 10–15 min. ^{1}H NMR data revealed the formation of the alkylated products by the appearance of the N–CH_3 peak at 3.11 and 2.89 ppm for compounds **3** and **9**, respectively. Reactions of compounds **3** and **9** with hydrazine monohydrate in refluxing ethanol resulted in cyclization and gave the respective pyrazolo [4,3-*c*][1,2]benzothiazine 5,5-dioxide scaffold (**4** and **10**). The IR stretching frequency for NH was observed at 3355 and 3368 cm^{-1}, and the C=N functionality was detected at 1594 and 1655 cm^{-1}. In the ^{1}H NMR spectra, the NH signal appeared as a broad singlet at 10.11 and 13.78 ppm for compounds **4** and **10**, respectively.

The following key intermediates were prepared by *N*-arylation of compounds **4** and **10** with 4-fluorobenzaldehyde in the presence of anhydrous K_2CO_3 and hexadecyltriphenylphosphonium bromide as phase transfer catalyst yielding 4-(3,4-dimethyl-5,5-dioxidobenzo[*e*]pyrazolo[4,3-*c*][1,2]thiazin-2(4*H*)-yl)benzaldehyde (**5**) and 4-(4-methyl-5,5-dioxido-3-phenylbenzo[*e*]pyrazolo[4,3-*c*][1,2]thiazin-2(4*H*)-yl)benzaldehyde (**11**), respectively. Absorption peaks at 1727 and 1737 cm^{-1} in the IR spectra and singlets at 10.03 and 10.23 ppm in the ^{1}H NMR spectra appeared for the aldehyde moiety of **5** and **11**, respectively. The ^{13}C NMR chemical shifts for the aldehyde carbons were detected at 190.9 and 191.0 ppm.

The final step was the condensation reaction of compounds **5** and **11** with a variety of substituted *o*-phenylenediamines in DMF in the presence of sodium metabisulfite, which resulted in the targeted compounds (Scheme 1), *i.e.* 2-[4-(substituted-1*H*-benzimidazol-2-yl)phenyl]-3,4-dimethyl-2,4-dihydropyrazolo[4,3-*c*][1,2] benzothiazine 5,5-dioxides (**6a**–**h**) and 2-[4-(substituted-1*H*-benzimidazol-2-yl)phenyl]-4-methyl-3-phenyl-2,4-dihydropyrazolo [4,3-*c*][1,2]benzothiazine 5,5-dioxides (**12a**–**h**). The formation of the benzimidazole structure was accomplished by the condensation reaction between the aldehyde and the diamine in the presence of sodium metabisulfite as an oxidizing agent. The spectroscopic data of the final compounds were in agreement with the proposed structures.

The last reaction step was similarly used for the preparation of structurally modified compounds (Scheme 2). In **6i** and **12i** or **6j** and **12j**, the benzimidazole core was exchanged for purine or benzoxazole, when **5** or **11** were reacted with pyrimidine-4,5-diamine or 2-aminophenol, respectively, in place of *o*-phenylenediamine. Moreover, starting from benzimidazole-5-carboxylic acid derivatives **6a** and **12a**, the corresponding ethyl esters (**6k** and **12k**) were prepared (Scheme 2).



Scheme 2. Synthesis of purine- and benzoxazole-based derivatives (**6i** and **12i**; **6j** and **12j**) and of functionalized benzimidazole derivatives (**6k** and **12k**).

3. *In vitro* AChE and BuChE inhibitory activity

In vitro inhibitory studies of the newly synthesized pyrazolo–benzothiazines derivatives were carried out on AChE from *Electrophorus electricus* (EeAChE) and BuChE from equine serum. Inhibition potencies of compounds expressed as IC_{50} values are shown in Table 1. These values suggest that most of these synthesized compounds are potent inhibitors of cholinesterases.

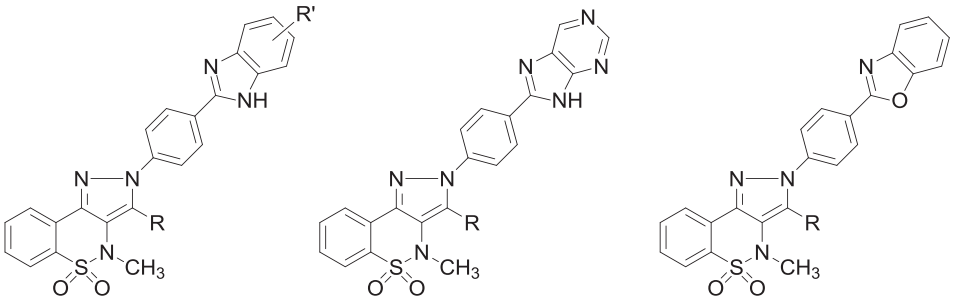
Compounds **12a**–**k** with phenyl substitution in R position mainly showed a lower potency against AChE and BuChE than the methyl substituted analogues **6a**–**k** (see, for example IC_{50} values of **6f** *versus* those of **12f**). This was, however, not the case for the representatives with an unsubstituted benzimidazole unit. The phenyl derivative **12d** was much more active on AChE than the methyl derivative **6d**, and both compounds exhibited the same IC_{50} for BuChE inhibition. Moreover, among the two compounds with the rather large and polar substituent at the benzimidazole core, *i.e.* ethoxycarbonyl, **12k** was more potent towards AChE than the analogous methyl derivative **6k**. Inhibitor **12k** exhibited moderate selectivity for AChE over BuChE (13 nM *versus* 180 nM). Also compound **12j** having a phenyl residue at position R and a 1,3-benzoxazol-2-yl group attached to the phenylene linker showed excellent AChE inhibition (IC_{50} 54 nM). Thus, we obtained four new inhibitors of AChE (**6f**, **12d**, **12j**, **12k**) with an activity similar to that of the reference compound donepezil.

Even more striking was the unexpected finding of the strong activity (and selectivity) of some of our compounds against BuChE (and over AChE). This was observed for the 1,3-benzoxazol-2-yl compound **6j** bearing a methyl group at position R. Inhibitor **6j** showed IC_{50} values of less than 20 nM and was thus much more potent at BuChE than donepezil. This inhibitor exhibited an at least 400-fold stronger inhibition of BuChE than of AChE.

4. Docking studies

The X-ray structures of human AChE (PDB ID 4BDT) and BuChE (PDB ID 4BDS) were selected for the docking study because available electric eel structures for AChE show low crystallographic resolutions (>4 Å) and structures of equine BuChE are not available at the moment. The active sites of AChE and BuChE show a high degree of similarity despite the replacement of residues Pro446, Tyr124 and Phe297 in the active site of AChE by residues Met437, Gln119 and Val288 in BuChE. In addition, residue Tyr337 in AChE is replaced by the smaller Ala328 in BuChE. Depending on

Table 1
Enzyme inhibition results for the 2,4-dihydropyrazolo[4,3-*c*][1,2]benzothiazine 5,5-dioxides **6a**–**k** and **12a**–**k**.



6a-i, k-m & 12a-i, k-m | **6i & 12i** | **6j & 12 j**

Compound	R	R′	IC_{50} ± SEM (μM) or (% inhibition at 500 μM)	
			AChE	BuChE
6a	Methyl	5-carboxy	1.13 ± 0.1	0.026 ± 0.001
6b	Methyl	5-chloro	1.02 ± 0.3	(38.7)
6c	Methyl	5-bromo	0.541 ± 0.01	0.049 ± 0.003
6d	Methyl	H	2.91 ± 0.5	0.043 ± 0.006
6e	Methyl	5-methyl	7.53 ± 0.9	0.041 ± 0.008
6f	Methyl	5,6-dimethyl	0.041 ± 0.008	0.273 ± 0.031
6g	Methyl	5-nitro	0.492 ± 0.04	(38.5)
6h	Methyl	5-methoxy	1.14 ± 0.1	6.92 ± 0.42
6i	Methyl	–	1.95 ± 0.1	(35.5)
6j	Methyl	–	6.92 ± 0.7	0.017 ± 0.002
6k	Methyl	5-ethoxycarbonyl	0.993 ± 0.09	(29.5)
12a	Phenyl	5-carboxy	6.11 ± 0.1	4.62 ± 0.61
12b	Phenyl	5-chloro	3.02 ± 0.1	9.94 ± 0.62
12c	Phenyl	5-bromo	6.9 ± 0.1	3.23 ± 0.26
12d	Phenyl	H	0.011 ± 0.004	0.043 ± 0.033
12e	Phenyl	5-methyl	0.122 ± 0.05	(31.8)
12f	Phenyl	5,6-dimethyl	3.82 ± 0.11	(34.1)
12g	Phenyl	5-nitro	7.94 ± 0.11	(30.7)
12h	Phenyl	5-methoxy	7.13 ± 0.12	(37.3)
12i	Phenyl	–	5.22 ± 0.11	1.33 ± 0.12
12j	Phenyl	–	0.054 ± 0.009	(36.5)
12k	Phenyl	5-ethoxycarbonyl	0.013 ± 0.004	0.18 ± 0.01
Neostigmine			22.2 ± 3.2	49.6 ± 6.11
Donepezil			0.032 ± 0.003	6.41 ± 0.34

the co-crystallized compounds, different X-ray structures revealed multiple side chain orientations of Tyr337. Therefore, the side chain of Tyr337 in AChE was treated flexibly in our docking study, while the corresponding residue Ala328 in BuChE has no side chain flexibility.

Based on the results of the biological evaluation, compound **12d** was selected in order to investigate the possible interactions between the inhibitor and the active sites of both enzymes. The upper part of Fig. 1 shows an overlay of the putative binding mode of the best dual AChE/BuChE inhibitor **12d** and the crystallographic ligand

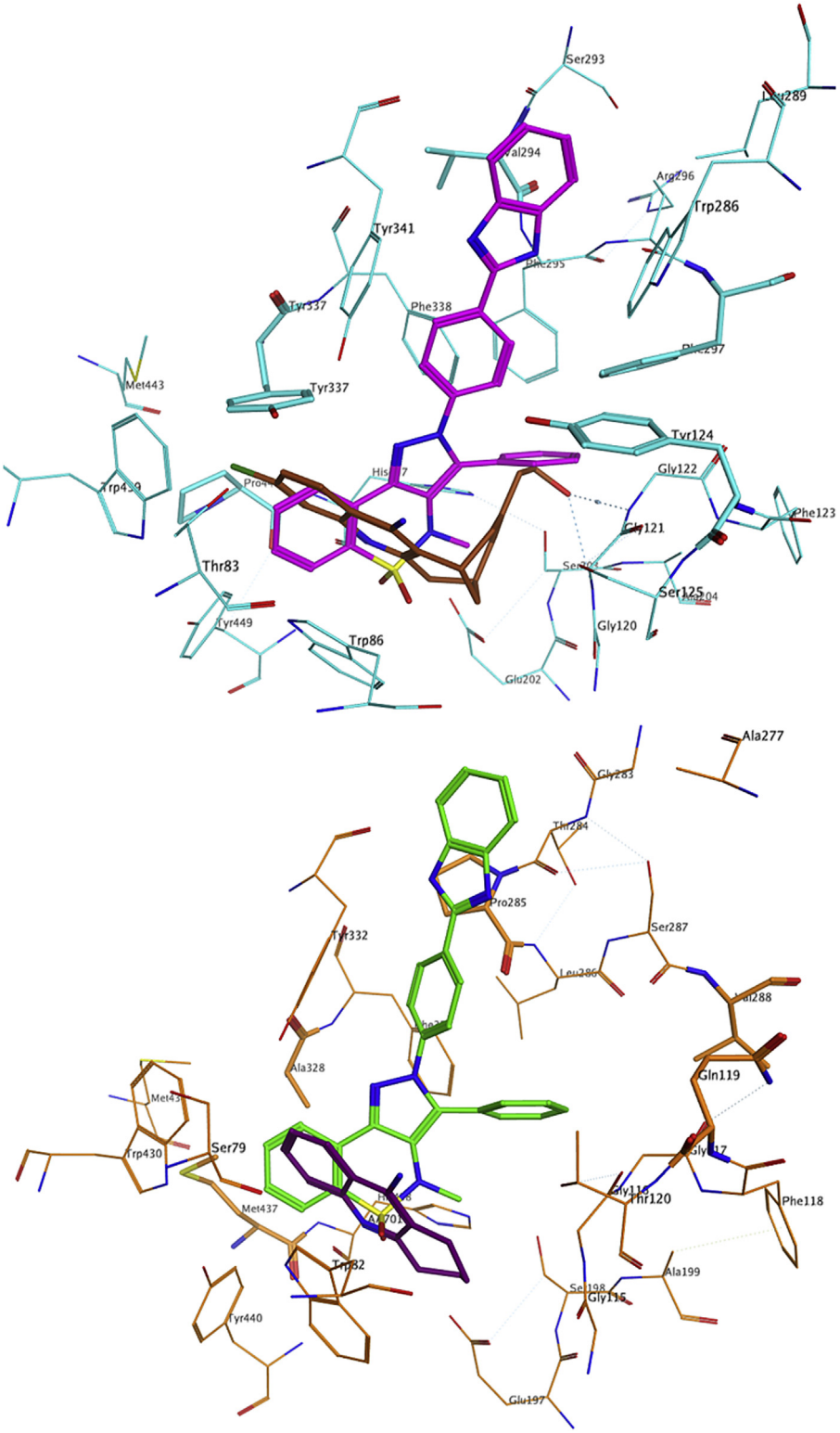


Fig. 1. Putative binding modes. Overlays of the docking poses of compound **12d** in the active sites of AChE (top, cyan) and BuChE (bottom, orange) with the crystallographic ligands huprine W (top, brown colour) and tacrine (bottom, purple colour) are shown. The active site is displayed in line representation and important residues which differ in AChE and BuChE are shown in stick representation. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

huprine W in the active site of AChE. The pyrazolobenzothiazine ring system of **12d** adopts a comparable position to huprine W and might interact with side chain atoms of several aromatic residues including Tyr337 and Trp86. The phenyl substituent at the pyrazol ring is directed into a hydrophobic subpocket formed by the residues Phe297, Ala204 and Phe123. The phenyl ring between the pyrazolobenzothiazine and the benzimidazole substructures is in π–π stacking interaction distance to residue Tyr341. The benzimidazole moiety is placed in the mid-gorge area of the binding site and might also form π–π interactions with the residue Trp286.

The lower part of Fig. 1 shows an overlay of the possible binding mode of compound **12d** in the active site of BuChE and the co-crystallized ligand tacrine. The predicted binding mode of **12d** in the active site of BuChE is very similar to the putative binding conformation of this compound in complex with AChE. In case of BuChE, the position of **12d** is slightly moved upwards (~1.5 Å) to the entrance of the pocket. The exchange of residue Tyr337 (AChE) to the smaller Ala328 (BuChE) allows a repositioning of **12d**, which enables a possible hydrogen bonding interaction of the benzimidazole substructure to the backbone carbonyl moiety of residue Tyr332.

Plausible binding conformations could be generated for the best dual inhibitor **12d** of AChE and BuChE, which are consistent with the presence of comparable potency of this compound against both enzymes. The orientation of the pyrazolobenzothiazine ring system correlates with the position of huprine W (AChE) and tacrine (BuChE) in both predicted binding conformations.

5. Conclusion

In this research article, we report on synthetic routes for the hybridization of pyrazolobenzothiazine scaffolds with benzimidazole ring systems. Structural diversity of the final compounds was ensured by using a range of substituted phenylenediamines and other bifunctional aromatic amines to also introduce purine and benzoxazole moieties. In light of the suggested role of BuChE in central cholinergic transmission, its altered expression in the AD brain, and its probable association with the development of neuropathologic changes, it was hypothesized that high BuChE activity would be detrimental in AD and that inhibiting this enzyme would be of clinical value along with AChE. Some of our inhibitors exhibit strong inhibition of BuChE and selectivity over AChE.

We identified compound **12d** (2-[4-(1*H*-benzimidazol-2-yl) phenyl]-4-methyl-3-phenyl-2,4-dihydropyrazolo[4,3-*c*][1,2]benzothiazine 5,5-dioxide) as a highly potent, dual-acting inhibitor of both cholinesterases. Computational analysis revealed plausible putative binding modes of the extended structure of **12d** along the active site gorge of AChE and BuChE. Inhibitor **12d** can be considered a promising lead for further investigations on this chemotype to develop disease-modifying drugs for the treatment of AD.

6. Experimental section

6.1. Chemicals

All the chemicals were purchased from Merck, Sigma/Aldrich or Fluka and used without purification. General melting points were obtained on a Gallenkamp melting point apparatus and were uncorrected. IR spectra were recorded in KBr pellets on Perkin Elmer infrared spectrophotometer. ^{1}H NMR spectra were recorded in DMSO-d_6, acetone-d_6 and $CDCl_3$ on a Bruker/XWIN NMR (300 and 400 MHz) apparatus, and TMS was used as internal standard (chemical shifts, δ in ppm). Mass spectra were recorded on a Jeol MS Route instrument. Ultrasonic mediated reactions were carried out in a Clifton Ultrasonic Bath (29 T2A, 300W, DU-4; Nickel Electro Ltd). Acetylcholinesterase (AChE, EC 3.1.1.7, type VI-S from electric eel), butylcholinesterase (BuChE, 3.1.1.8, from equine serum), acetylthiocholine iodide (ATCI), *S*-butyrylthiocholine chloride (BTCCl), 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB), neostigmine methylsulphate and dimethylsulfoxide (DMSO) and donepezil hydrochloride were purchased from Sigma–Aldrich (St. Louis, MO, USA).

6.1.1. General procedure for the synthesis of 2-(2-oxopropyl)-1,2-benzisothiazol-3(2H)-one 1,1-dioxide (N-acetonylsaccharin) and 2-(2-oxo-2-phenylethyl)-1,2-benzisothiazol-3(2H)-one 1,1-dioxide (N-phenacylsaccharin) (**1** & **7**)

Saccharin sodium salt (0.222 mol) was mixed with chloroacetone (0.25 mol) or phenacyl bromide (0.222 mol) for compounds **1** and **7** respectively in dried DMF with continuous stirring. The reaction mixture was heated for 3 h at 110 °C under inert atmosphere, cooled to ambient temperature and poured on crushed ice. The precipitate was filtered, washed with cold water and recrystallized from ethanol. The reactions were performed under 30W microwave irradiation for 25 min and in ultrasonic bath for 40 min.

*6.1.1.1. 2-(2-Oxopropyl)-1,2-benzisothiazol-3(2H)-one 1,1-dioxide (N-acetonylsaccharin, **1**).* Off white solid; yield: 86%; m.p.: 112–114 °C; FT-IR (KBr): 1717, 1584, 1355, 1185 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.24 (3H, s, C–CH_3), 4.77 (2H, s, N–CH_2) 7.57 (1H, d, J = 7.8 Hz, Ar–*H*), 7.66 (1H, t, J = 7.4 Hz, Ar–*H*), 7.96 (2H, d, J = 7.6 Hz, Ar–*H*); MS *m*/*z*: 239.0 [M$^+$].

*6.1.1.2. 2-(2-Oxo-2-phenylethyl)-1,2-benzisothiazol-3(2H)-one 1,1-dioxide (N-phenacylsaccharin, **7**).* Off white solid; yield: 83%; FT-IR (KBr): 1715, 1345, 1165 cm^{-1}; ^{1}H NMR (DMSO-d_6) (400 MHz) δ: 5.15 (2H, s, *N*–CH_2), 7.47 (2H, t, J = 7.4 Hz, Ar–*H*), 7.59 (1H, t, J = 7.4 Hz, Ar–*H*), 7.81–7.90 (2H, m, Ar–*H*), 7.92 (1H, d, J = 6.6 Hz, Ar–*H*), 7.94 (2H, d, J = 6.8 Hz, Ar–*H*), 8.07 (1H, d, J = 7.8 Hz, Ar–*H*); ^{13}C NMR (DMSO-d_6) δ: 51.3 (CH_2), 123.2 (Ar–C), 124.0 (Ar–C), 126.3 (Ar–C), 129.2 (2C, Ar), 129.4 (2C, Ar), 133.5 (Ar–C), 134.8 (Ar–C), 135.3 (Ar–C), 137.0 (Ar–C), 141.1 (Ar–C), 169.5 (C=O), 191.3 (Ar–CO); MS *m*/*z*: ES–; 301.1 (M$^+$), +ES; 324.04 (M$^+$ + Na$^+$).

6.1.2. General procedure for the synthesis of 3-acetyl-4-hydroxy-2H-1,2-benzothiazine 1,1-dioxide 3-benzoyl-4-hydroxy-2H-1,2-benzothiazine 1,1-dioxide (**2** & **8**)

Fresh sodium methoxide was first prepared by refluxing the sodium metal (5.34 g, 0.232 mol) in 60 mL of freshly dried methanol under inert atmosphere maintained by N_2 gas. Subsequently, *N*-acetonylsaccharine (5.0 g, 20.9 mmol) (**1**) or *N*-phenacylsaccharin (**7**) (10.0 g, 0.232 mmol) was added to the refluxing methanolic solution. The reaction mixture immediately turned to orange-red colour. The mixture was further refluxed for 20 min and then cooled to room temperature and poured to ice cold 10% HCl solution. The resulting white precipitate was filtered, washed with excess water and dried. (Caution: atmosphere should be inert otherwise traces of moisture could cause a drastic decrease in yield).

*6.1.2.1. 3-Acetyl-4-hydroxy-2H-1,2-benzothiazine 1,1-dioxide (**2**).* Yellowish brownish amorphous material; yield: 80%; m.p.: 155 °C; IR (KBr): 3472, 3251, 1597, 1584, 1355, 1177 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.26 (3H, s, C–CH_3), 5.87 (1H, s, SO_2NH), 7.57 (1H, t, J = 7.2 Hz, Ar–*H*), 7.69 (1H, t, J = 7.4 Hz, Ar–*H*), 7.97 (2H, t, J = 8.0 Hz, Ar–*H*), 14.53 (1H, s, OH); ^{13}C NMR (DMSO-d_6) δ: 21.5 (C–CH_3), 69.2 (C-3, thiazine), 122.5 (Ar–C), 123.4 (Ar–C), 124.4 (Ar–C), 127.2 (Ar–C), 129.2 (Ar–C), 133.4 (Ar–C), 162.7 (C-4, thiazine), 191.2 (C=O); MS *m*/*z*: 239.03; [M$^+$]. HR-MS (ES+) calcd for $C_{10}H_9NO_4S$, 239.0252; found, 240.0326 (M + H$^+$).

*6.1.2.2. 3-Benzoyl-4-hydroxy-2H-1,2-benzothiazine 1,1-dioxide (**8**).* White crystalline solid; yield: 82%; m.p.: 156 °C; FT-IR (KBr): 3353, 3133, 1625, 1358, 1157 cm^{-1}; ^1H NMR (DMSO-d_6) (400 MHz) δ: 5.79 (1H, s, SO_2NH), 7.61 (1H, t, $J = 7.8$ Hz, Ar–*H*), 7.71 (1H,d, $J = 7.8$ Hz, Ar–*H*), 7.95–7.99 (5H, m, Ar–*H*), 8.15 (2H, t, $J = 4.6$ Hz, Ar–*H*), 14.67 (1H, s, O–H); ^{13}C NMR (DMSO-d_6) δ: 110.1 (C-3, thiazine), 122.9 (Ar–C), 127.1 (Ar–C), 128.1 (Ar–C), 128.3 (Ar–C), 129.2 (Ar–C), 129.4 (2C, Ar), 132.2 (Ar–C), 132.3 (Ar–C), 134.0 (Ar–C), 137.1 (2C, Ar), 167.6 (C-4, thiazine), 191.1 (C=O); MS *m*/*z*: (ES−) 301.07 (M^+), (+ES) 302.06 ($M^+ + H^+$).

*6.1.3. General procedure for the synthesis of 3-acetyl-4-hydroxy-2-methyl-2H-1,2-benzothiazine 1,1-dioxide and 3-benzoyl-4-hydroxy-2-methyl-2H-1,2-benzothiazine 1,1-dioxide (**3** & **9**)*

3-Acetyl-4-hydroxy-2*H*-1,2-benzothiazine 1,1-dioxide (**2**) (10.0 g, 42 mmol) or 3-benzoyl-4-hydroxy-2*H*-1,2-benzothiazine 1,1-dioxide (**8**) (10.0 g, 33.2 mmol) and aqueous sodium hydroxide (2.66 g in 5 mL water) were mixed in 50 mL of acetone. The mixture was allowed to stir at ambient temperature for 10 min. Dimethyl sulphate (8.06 mL, 64 mmol) was added dropwise. The mixture was placed in ultrasonic media for 15 min at ambient temperatures. The resulting precipitate was obtained after addition of cold dilute HCl (20 mL; 5%) and was filtered, washed with excess water and then dried. The reaction was performed at room temperature for 30–45 min.

*6.1.3.1. 3-Acetyl-4-hydroxy-2-methyl-2H-1,2-benzothiazine 1,1-dioxide (**3**).* White crystalline solid; yield: 89%; m.p.: 152 °C; FT-IR (KBr): 3475, 1601, 1588, 1349, 1191 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 2.24 (3H, s, C–CH_3), 3.11 (3H, s, N–CH_3), 7.56 (1H, t, $J = 7.4$ Hz, Ar–*H*), 7.69 (1H, t, $J = 7.0$ Hz, Ar–*H*), 7.94 (2H, t, $J = 7.5$ Hz, Ar–*H*), 12.34 (1H, s, OH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 21.5 (C–CH_3), 38.7 (N–CH_3), 117.9 (Ar–C), 123.4 (Ar–C), 124.4 (Ar–C), 127.2 (Ar–C), 129.2 (Ar–C), 133.4 (Ar–C), 136.7 (Ar–C), 161.2 (C-4, thiazine), 192.02 (C=O); MS *m*/*z*: 253.0 [M^+]. HR-MS (ES+) calcd for $C_{11}H_{11}NO_4S$, 253.0409; found, 254.0471 ($M + H^+$).

*6.1.3.2. 3-Benzoyl-4-hydroxy-2-methyl-2H-1,2-benzothiazine 1,1-dioxide (**9**).* Light yellow crystalline solid; yield: 85%; m.p.: 147–148 °C; FT-IR (KBr): 3470, 2970, 1610, 1586, 1357, 1184 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 2.89 (3H, s, N–CH_3), 7.59 (2H, t, $J = 7.6$ Hz, Ar–*H*), 7.69 (1H,d, $J = 7.8$ Hz, Ar–*H*), 7.73–7.77 (2H, dd, $J_1 = 2.8$ Hz, $J_2 = 8.4$ Hz, Ar–*H*), 7.94–7.97 (3H, m, Ar–*H*), 8.17 (1H, t, $J = 4.6$ Hz, Ar–*H*), 14.69 (1H, s, O–*H*); ^{13}C NMR (DMSO-d_6) δ: 39.8 (N–CH_3), 121.9 (C-3, thiazine), 123.2 (Ar–C), 124.9 (Ar–C), 124.7 (Ar–C), 127.2 (Ar–C), 128.6 (Ar–C), 129.2 (Ar–C), 130.1 (Ar–C), 131.0 (Ar–C), 133.0 (Ar–C), 134.2 (Ar–C), 136.5 (Ar–C), 138.2 (Ar–C), 167.2 (C-4, thiazine), 190.0 (C=O); MS *m*/*z*: 315.3 [M^+].

*6.1.4. General procedure for the synthesis of 3,4-dimethyl-2,4-dihydropyrazolo[4,3-c][1,2]benzothiazine 5,5-dioxide and 4-methyl-3-phenyl-2,4-dihydrobenzo[e]pyrazolo[4,3-c][1,2]thiazine 5,5-dioxide (**4** & **10**)*

A mixture of 1-(4-hydroxy-2-methyl-1,1-dioxido-2*H*-1,2-benzothiazin-3-yl)ethanone (3) (5.0 g, 20.9 mmol) or 3-benzoyl-4-hydroxy-2-methyl-2*H*-1,2-benzothiazine 1,1-dioxide (9) (5.0 g, 13.08 mmol) and hydrazine monohydrate (4.9 mL, 99 mmol) in 50 mL ethanol was refluxed for 3–4 h. Then the unreacted hydrazine monohydrate and ethanol was removed under vacuum. The crude product was treated with ice cold water. The resulting precipitate was filtered and recrystallized from ethanol.

*6.1.4.1. 3,4-Dimethyl-2,4-dihydropyrazolo[4,3-c][1,2]benzothiazine 5,5-dioxide (**4**).* Brownish yellow solid; yield: 81%; m.p.: 231 °C; FT-IR (KBr): 3355, 1594, 1315, 1156 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 2.29 (3H, s, C–CH_3), 3.12 (3H, s, N–CH_3), 7.55 (1H, t, $J = 7.6$ Hz, Ar–*H*), 7.63 (1H, d, $J = 7.4$ Hz, Ar–*H*), 7.97 (2H, t, $J = 8.0$ Hz, Ar–*H*), 10.11 (1H, s, NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 8.6 (C–CH_3), 38.8 (N–CH_3), 121.8 (Ar–C), 123.3 (Ar–C), 124.2 (Ar–C), 128.8 (Ar–C), 129.3 (Ar–C), 131.1 (Ar–C), 132.9 (Ar–C), 133.2 (C=N, Ar–C), 134.5 (C=C–SO_2); MS *m*/*z*: 249.1 (M^+). HR-MS (ES+) calcd for $C_{11}H_{11}N_3O_2S$, 249.0572; found, 250.0650 ($M + H^+$).

*6.1.4.2. 4-Methyl-3-phenyl-2,4-dihydrobenzo[e]pyrazolo[4,3-c][1,2]thiazine 5,5-dioxide (**10**).* Off white crystalline; yield: 87%; FT-IR (KBr): 3368, 2965, 1655, 1337, 1165 cm^{-1}; ^1H NMR (DMSO-d_6) (400 MHz) δ: 2.91 (3H, s, N–CH_3), 7.49 (1H, t, $J = 7.8$ Hz, Ar–*H*), 7.61 (2H, q, $J = 8.4$ Hz, Ar–*H*), 7.64–7.71 (1H, dq, $J_1 = 1.2$ Hz, $J_2 = 7.6$ Hz, $J_3 = 11.2$ Hz, $J_4 = 16.8$ Hz, Ar–*H*), 7.73–7.79 (1H, dd, $J_1 = 1.5$ Hz, $J_2 = 8.4$ Hz, Ar–*H*), 7.94–8.01 (3H, m, Ar–*H*), 8.17 (1H, d, $J = 7.6$ Hz, Ar–*H*), 13.78 (1H, s, N–*H*); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 39.8 (N–CH_3), 122.2 (Ar–C), 124.2 (Ar–C), 124.7 (Ar–C), 124.9 (Ar–C), 125.8 (Ar–C), 125.9 (Ar–C), 127.2 (Ar–C), 128.6 (Ar–C), 130.1 (Ar–C), 130.3 (Ar–C), 130.4 (Ar–C), 134.2 (Ar–C), 134.4 (C=C–SO_2), 138.2 (Ar–C), 147.4 (N=C, Ar–C); MS *m*/*z*: 312.4 (ES+), HR-MS (ES+) calcd for $C_{16}H_{13}N_3O_2S$, 311.0728; found, 312.0801 ($M + H^+$).

*6.1.5. General procedure for the synthesis of 4-(3,4-dimethyl-5,5-dioxidobenzo[e]pyrazolo[4,3-c][1,2]thiazin-2(4H)-yl)benzaldehyde (**5**) and 4-(4-methyl-5,5-dioxido-3-phenylbenzo[e]pyrazolo[4,3-c][1,2]thiazin-2(4H)-yl)benzaldehyde (**11**)*

3,4-Dimethyl-2,4-dihydropyrazolo[4,3-*c*][1,2]benzothiazine 5,5-dioxide (**4**) (12.50 g; 50.0 mmol) or 4-methyl-3-phenyl-2,4-dihydrobenzo[*e*]pyrazolo[4,3-*c*][1,2]thiazine 5,5-dioxide (**10**) (15.55 g; 50.0 mmol), 4-fluorobenzaldehyde (7.44 g; 60.0 mmol) and anhydrous K_2CO_3 (8.30 g; 60.0 mmol) were dissolved in dried DMF (25 mL) followed by the addition of hexadecyltriphenylphosphonium bromide (2.54 g; 5.0 mmol). The reaction mixture was refluxed under the nitrogen atmosphere for 40 min. Ice cold water was added and the resulting pale-yellow precipitate was filtered, washed with excess water and recrystallized from ethanol.

*6.1.5.1. 4-(3,4-Dimethyl-5,5-dioxidobenzo[e]pyrazolo[4,3-c][1,2]thiazin-2(4H)-yl)benzaldehyde (**5**).* Yellowish solid; yield: 73%; m.p.: 231 °C; FT-IR (KBr): 3097, 2994, 2927, 2836, 2771, 1727, 1598, 1445, 1359, 1244, 1161, 821, 725 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 2.49 (3H, s, C–CH_3), 3.09 (3H, s, N–CH_3), 7.52 (1H, t, $J = 7.5$ Hz, Ar–*H*), 7.62–7.70 (4H, dd, $J_1 = 6.3$ Hz, $J_2 = 12.9$ Hz, Ar–*H*), 7.91 (1H, d, $J = 6.6$ Hz, Ar–*H*), 7.95 (1H, d, $J = 8.4$ Hz, Ar–*H*), 8.04 (1H, d, $J = 7.2$ Hz, Ar–*H*), 10.03 (1H, s, CH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 11.0 (C–CH_3), 40.0 (N–CH_3), 124.3 (Ar–C), 124.8 (Ar–C), 124.9 (2C) (Ar–C), 125.5 (Ar–C), 127.8 (Ar–C),129.3 (2C) (Ar–C), 130.9 (Ar–C), 132.6 (Ar–C), 133.0 (Ar–C), 133.6 (Ar–C), 135.5 (Ar–C), 139.7 (Ar–C), 144.0 (N=C, Ar–C), 190.9 (–HC=O); MS *m*/*z*: (ES−) 353 (M^+), (ES+) 354.1 ($M + H^+$); HR-MS(+ES) calcd for $C_{18}H_{15}N_3O_3S$, 353.0834; found, 354.0907 ($M + H^+$).

*6.1.5.2. 4-(4-Methyl-5,5-dioxido-3-aphenylbenzo[e]pyrazolo[4,3-c][1,2]thiazin-2(4H)-yl)benzaldehyde (**11**).* Yellowish solid; yield: 69%; FT-IR (KBr): 3079, 2987, 2847, 2779, 1737, 1701, 1598, 1456, 1362, 1204, 1162, 824, 723 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 3.23 (3H, s, N–CH_3), 7.43–7.57 (2H, m, Ar–*H*), 7.67 (1H, t, $J = 7.5$ Hz, Ar–*H*), 7.75–7.83 (4H, dd, $J_1 = 9.0$ Hz, $J_2 = 15.4$ Hz, Ar–*H*), 7.91 (1H, d, $J = 7.8$ Hz, Ar–*H*), 7.97–8.05 (4H, dd, $J_1 = 7.2$ Hz, $J_2 = 14.7$ Hz, Ar–*H*), 8.13 (1H, d, $J = 7.2$ Hz, Ar–*H*), 10.23 (1H, s, CH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 38.7 (N–CH_3), 124.3 (2C) (Ar–C), 124.7 (Ar–C), 125.1 (Ar–C), 125.2 (Ar–C), 126.9 (Ar–C), 127.1 (Ar–C), 127.8 (Ar–C), 128.3 (Ar–C), 129.2 (Ar–C), 129.4 (Ar–C), 129.9 (2C) (Ar–C), 130.5 (Ar–C), 131.0 (Ar–C), 133.3 (Ar–C), 135.6 (Ar–C), 137.9 (Ar–C), 139.1

(Ar–C), 140.2 (Ar–C), 143.0 (N=C, Ar–C), 191.0 (–HC=O); MS *m/z*: 416.1 (ES+, M + H^+); HR-MS (ES+) calcd for $C_{23}H_{17}N_3O_3S$, 415.0991; found, 416.1064 (M + H^+).

*6.1.6. General procedure for the synthesis of pyrazolo[4,3-c][1,2] benzothiazine 5,5-dioxides (**6a**–**k** and **12a**–**k**) and purine- and benzoxazole-containing derivatives (**6i** and **12i**; **6j** and **12j**)*

4-(3,4-Dimethyl-5,5-dioxidobenzo[*e*]pyrazolo[4,3-*c*][1,2]thiazin-2(4*H*)-yl)benzaldehyde (**5**) (0.01 mol) or 4-(4-methyl-5,5-dioxido-3-phenylbenzo[*e*]pyrazolo[4,3-*c*][1,2]thiazin-2 (4*H*)yl)benzaldehyde (**11**) (0.01 mol) and sodium metabisulfite (0.01 mol) were dissolved in dried DMF (20 mL) and stirred at room temperature for 15–20 min. Then, substituted *o*-phenylenediamine or pyrimidine-4,5-diamine (for **6i** and **12i**) or 2-aminophenol (for **6j** and **12j**) (0.011 mol) was added and stirred at 135–140 °C for 6–8 h depending on the reactivity of the aromatic amine. The reaction was monitored by TLC. After completion, the reaction mixture was allowed to cool at room temperature and poured into ice cold water. The resulting precipitate was filtered, washed with water and recrystallized from ethanol.

*6.1.6.1. 2-[4-(5-Carboxy-1H-benzimidazol-2-yl)phenyl]-3,4-dimethyl-2,4-dihydropyrazolo[4,3-c][1,2]benzothiazine 5,5-dioxide (**6a**).* Light brown powder; Yield: 87%; FT-IR (KBr): 3645, 3463, 3205, 3026, 2969, 1738, 1652, 1495, 1434, 1372, 1229, 1167, 1063, 847, 770, 745 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 2.51 (3H, s, C–CH_3), 3.06 (3H, s, N–CH_3), 7.70 (2H, t, J = 8.1 Hz, Ar–*H*), 7.82–7.96 (5H, m, Ar–*H*), 8.08 (1H, d, J = 7.5 Hz, Ar–*H*), 8.22 (1H, s, Ar–*H*), 8.41 (2H, d, J = 8.4 Hz, Ar–*H*), 13.29 (1H, s, NH); ^{13}C NMR (acetone-d_6, 100 MHz) δ: 10.9 (C–CH_3), 40.3 (N–CH_3), 122.4 (Ar–C), 123.7 (Ar–C), 124.8 (Ar–C), 125.1 (Ar–C), 125.5 (Ar–C), 125.8 (Ar–C), 126.0 (Ar–C), 128.4 (Ar–C), 128.7 (Ar–C), 128.9 (Ar–C), 129.1 (Ar–C), 129.8 (Ar–C), 130.2 (Ar–C), 130.5 (Ar–C), 131.5 (Ar–C), 132.5 (Ar–C), 133.8 (Ar–C), 134.0 (Ar–C), 135.0 (Ar–C), 139.8 (Ar–C), 141.9 (N=C, Ar–C), 152.9 (N=C–N, Ar–C), 168.1 (C=O); MS *m/z*: 484 (ES–), 486 (ES+); HR-MS (ES–) calcd for $C_{25}H_{19}N_5O_4S$, 484.1158; found, 484.1073.

*6.1.6.2. 2-[4-(5-Chloro-1H-benzimidazol-2-yl)phenyl]-3,4-dimethyl-2,4 dihydropyrazolo[4,3-c][1,2]benzothiazine 5,5-dioxide (**6b**).* Off white powder; Yield: 82%; FT-IR (KBr): 3341, 3290, 3068, 2926, 1739, 1660, 1609, 1581, 1470, 1343, 1232, 1169, 1061, 848, 772, 746 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 2.51 (3H, s, C–CH_3), 3.06 (3H, s, N–CH_3), 7.24–7.27 (1H, dd, J_1 = 1.8 Hz, J_2 = 8.7 Hz, Ar–*H*), 7.63–7.73 (3H, m, Ar–*H*), 7.83 (1H, d, J = 7.8 Hz, Ar–*H*), 7.88 (2H, d, J = 8.4 Hz, Ar–*H*), 7.94 (1H, d, J = 7.8 Hz, Ar–*H*), 8.08 (1H, d, J = 7.5 Hz, Ar–*H*), 8.36 (2H, d, J = 10.2 Hz, Ar–*H*), 13.28 (1H, s, NH); ^{13}C NMR (acetone-d_6, 100 MHz) δ: 10.8 (C–CH_3), 40.3 (N–CH_3), 122.4 (Ar–C), 123.8 (Ar–C), 124.8 (Ar–C), 125.2 (Ar–C), 125.5 (Ar–C), 125.8 (Ar–C), 126.1 (Ar–C), 128.5 (Ar–C), 128.7 (Ar–C), 128.8 (Ar–C), 129.1 (Ar–C), 129.8 (Ar–C), 130.2 (Ar–C), 130.6 (Ar–C), 131.5 (Ar–C), 132.4 (Ar–C), 133.8 (Ar–C), 134.0 (Ar–C), 134.9 (Ar–C), 139.8 (Ar–C), 141.8 (N=C, Ar–C), 152.9 (N=C–N, Ar–C); MS *m/z*: 474 (ES–), 476 (ES+); HR-MS (ES–) calcd for $C_{24}H_{18}ClN_5O_2S$, 475.0870; found, 474.0805.

*6.1.6.3. 2-[4-(5-Bromo-1H-benzimidazol-2-yl)phenyl]-3,4-dimethyl-2,4-dihydropyrazolo[4,3-c][1,2]benzothiazine 5,5-dioxide (**6c**).* Brown powder; Yield: 84%; FT-IR (KBr): 3277, 3067, 2969, 2925, 1655, 1601, 1493, 1435, 1375, 1169, 1062, 845, 773, 745 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 2.51 (3H, s, C–CH_3), 3.06 (3H, s, N–CH_3), 7.35–7.38 (1H, dd, J_1 = 1.8 Hz, J_2 = 8.7 Hz, Ar–*H*), 7.59 (1H, d, J = 8.7 Hz, Ar–*H*), 7.71 (1H, t, J = 7.8 Hz, Ar–*H*), 7.83 (2H, d, J = 6.3 Hz, Ar–*H*), 7.88 (2H, d, J = 8.7 Hz, Ar–*H*), 7.94 (1H, d, J = 7.8 Hz, Ar–*H*), 8.08 (1H, d, J = 7.5 Hz, Ar–*H*), 8.36 (2H, d, J = 8.7 Hz, Ar–*H*), 13.29 (1H, s, NH); ^{13}C NMR (acetone-d_6, 100 MHz) δ: 10.8 (C–CH_3), 40.3 (N–CH_3), 122.3 (Ar–C), 123.6 (Ar–C), 124.8 (Ar–C), 125.1 (Ar–C), 125.5 (Ar–C), 125.8 (Ar–C), 126.0 (Ar–C), 128.3 (Ar–C), 128.5 (Ar–C), 128.9 (Ar–C), 129.0 (Ar–C), 129.6 (Ar–C), 130.1 (Ar–C), 130.2 (Ar–C), 131.5 (Ar–C), 132.4 (Ar–C), 133.8 (Ar–C), 134.0 (Ar–C), 135.0 (Ar–C), 139.8 (Ar–C), 141.8 (N=C, Ar–C), 162.9 (N=C–N, Ar–C); MS *m/z*: 518 (ES–), 520 (M + 2), 522 (ES+); HR-MS (ES–) calcd for $C_{24}H_{18}BrN_5O_2S$, 519.0365; found, 518.0286.

*6.1.6.4. 2-[4-(1H-Benzimidazol-2-yl)phenyl]-3,4-dimethyl-2,4-dihydropyrazolo[4,3-c][1,2]benzothiazine 5,5-dioxide (**6d**).* Light brown powder; Yield: 83%; FT-IR (KBr): 3272, 2978, 2928, 1753, 1660, 1601, 1500, 1439, 1376, 1169, 1064, 847, 768, 744 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ: 2.51 (3H, s, C–CH_3), 3.06 (3H, s, N–CH_3), 7.24 (2H, t, J = 6.9 Hz, Ar–*H*), 7.57 (1H, d, J = 7.8 Hz, Ar–*H*), 7.71 (2H, t, J = 7.4 Hz, Ar–*H*), 7.82–7.88 (3H, m, Ar–*H*), 7.94 (1H, d, J = 7.8 Hz, Ar–*H*), 8.08 (1H, d, J = 7.5 Hz, Ar–*H*), 8.37 (2H, d, J = 8.7 Hz, Ar–*H*), 13.07 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz) δ: 10.8 (C–CH_3), 40.3 (N–CH_3), 122.4 (Ar–C), 123.4 (Ar–C), 124.8 (Ar–C), 125.1 (Ar–C), 125.5 (Ar–C), 125.8 (Ar–C), 127.0 (Ar–C), 128.3 (Ar–C), 128.6 (Ar–C), 128.9 (Ar–C), 129.1 (Ar–C), 129.8 (Ar–C), 130.2 (Ar–C), 130.5 (Ar–C), 131.2 (Ar–C), 132.5 (Ar–C), 133.7 (Ar–C), 134.0 (Ar–C), 134.9 (Ar–C), 139.7 (Ar–C), 141.4 (N=C, Ar–C), 151.3 (N=C–N, Ar–C); MS *m/z*: 440 (ES–), 442 (ES+); HR-MS (ES–) calcd for $C_{24}H_{19}N_5O_2S$, 441.1259; found, 440.1179.

*6.1.6.5. 2-[4-(5-Methyl-1H-benzimidazol-2-yl)phenyl]-3,4-dimethyl-2,4-dihydropyrazolo[4,3-c][1,2]benzothiazine 5,5-dioxide (**6e**).* Light brown powder; Yield: 85%; FT-IR (KBr): 3272, 2969, 2921, 1739, 1609, 1507, 1439, 1375, 1170, 1064, 849, 773, 746 cm^{-1}; ^1H NMR (acetone-d_6, 400 MHz) δ: 2.35 (3H, s, Ar–CH_3), 2.47 (3H, s, C–CH_3), 3.00 (3H, s, N–CH_3), 6.94–6.97 (1H, dd, J_1 = 1.2 Hz, J_2 = 8.0 Hz, Ar–*H*), 7.30 (1H, s, Ar–*H*), 7.40 (1H, d, J = 8.0 Hz, Ar–*H*), 7.56–7.60 (1H, dt, J_1 = 1.2 Hz, J_2 = 7.6 Hz, J_3 = 15.6 Hz, Ar–*H*), 7.68–7.72 (1H, dt, J_1 = 1.2 Hz, J_2 = 7.6 Hz, J_3 = 16.4 Hz, Ar–*H*), 7.71–7.74 (2H, dd, J_1 = 2.4 Hz, J_2 = 6.8 Hz, Ar–*H*), 7.78 (1H, q, J = 4.0 Hz, Ar–*H*), 7.83 (1H, d, J = 8.0 Hz, Ar–*H*), 7.99 (1H, d, J = 8.4 Hz, Ar–*H*), 8.34 (1H, d, J = 8.4 Hz, Ar–*H*); ^{13}C NMR (acetone-d_6, 100 MHz) δ: 10.8 (C–CH_3), 20.7 (Ar–CH_3), 40.3 (N–CH_3), 122.4 (Ar–C), 123.4 (Ar–C), 124.8 (Ar–C), 125.1 (Ar–C), 125.5 (Ar–C), 125.8 (Ar–C), 126.0 (Ar–C), 128.2 (Ar–C), 128.6 (Ar–C), 128.1 (Ar–C), 129.2 (Ar–C), 129.8 (Ar–C), 130.2 (Ar–C), 130.4 (Ar–C), 131.5 (Ar–C), 132.5 (Ar–C), 133.7 (Ar–C), 133.9 (Ar–C), 135.0 (Ar–C), 139.8 (Ar–C), 141.9 (N=C, Ar–C), 152.0 (N=C–N, Ar–C); MS *m/z*: 454 (ES–), 456 (ES+); HR-MS (ES–) calcd for $C_{25}H_{21}N_5O_2S$, 455.1416; found, 454.1358.

*6.1.6.6. 2-[4-(5,6-Dimethyl-1H-benzimidazol-2-yl)phenyl]-3,4-dimethyl-2,4-dihydropyrazolo[4,3-c][1,2]benzothiazine 5,5-dioxide (**6f**).* Light yellow powder; Yield: 90%; FT-IR (KBr): 3021, 3004, 2968, 1742, 1608, 1505, 1441, 1375, 1216, 1169, 1061, 849, 771, 746 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ: 2.31 (6H, s, 2XAr–CH_3), 2.36 (3H, s, C–CH_3), 3.06 (3H, s, N–CH_3), 7.45 (2H, s, Ar–*H*), 7.54 (3H, d, J = 7.4 Hz, Ar–*H*), 7.64 (1H, t, J = 7.8 Hz, Ar–*H*), 7.94 (1H, d, J = 7.5 Hz, Ar–*H*), 8.03 (1H, d, J = 7.8 Hz, Ar–*H*), 8.18 (2H, d, J = 7.5 Hz, Ar–*H*); ^{13}C NMR (acetone-d_6, 100 MHz) δ: 10.8 (C–CH_3), 20.4 (Ar–CH_3), 29.9 (Ar–CH_3), 40.3 (N–CH_3), 122.4 (Ar–C), 123.5 (Ar–C), 124.8 (Ar–C), 125.1 (Ar–C), 125.5 (Ar–C), 125.8 (Ar–C), 125.9 (Ar–C), 128.1 (Ar–C), 128.6 (Ar–C), 128.9 (Ar–C), 129.2 (Ar–C), 129.8 (Ar–C), 130.1 (Ar–C), 130.5 (Ar–C), 131.4 (Ar–C), 132.3 (Ar–C), 133.7 (Ar–C), 133.9 (Ar–C), 134.9 (Ar–C), 139.7 (Ar–C), 141.2 (N=C, Ar–C), 150.4 (N=C–N, Ar–C); MS *m/z*: 468 (ES–), 470 (ES+); HR-MS (ES–) calcd for $C_{26}H_{23}N_5O_2S$, 469.1572; found, 468.1485.

*6.1.6.7. 2-[4-(5-Nitro-1H-benzimidazol-2-yl)phenyl]-3,4-dimethyl-2,4-dihydro pyrazolo [4,3-c][1,2] benzothiazine 5,5-dioxide (**6g**).* Yellow powder; Yield: 85%; FT-IR (KBr): 3113, 3095, 2929, 1655, 1600, 1494, 1438, 1374, 1243, 1170, 1064, 1006, 849, 772, 745 cm^{-1};

^{1}H NMR (acetone-d_6, 400 MHz) δ: 2.48 (3H, s, C–CH_3), 2.99 (3H, s, N–CH_3), 7.20 (1H, t, J = 5.2 Hz, Ar–*H*), 7.47 (1H, d, J = 2.4 Hz, Ar–*H*), 7.57–7.61 (1H, dt, J_1 = 1.2 Hz, J_2 = 7.6 Hz, J_3 = 15.2 Hz, Ar–*H*), 7.69–7.73 (1H, dt, J_1 = 1.2 Hz, J_2 = 7.6 Hz, J_3 = 15.2 Hz, Ar–*H*), 7.80 (2H, t, J = 8.4 Hz, Ar–*H*), 7.99 (1H, d, J = 7.6 Hz, Ar–*H*), 8.06–8.09 (2H, dd, J_1 = 2.0 Hz, J_2 = 8.8 Hz, Ar–*H*), 8.35 (1H, d, J = 8.4 Hz, Ar–*H*), 8.43 (1H, s, Ar–*H*), 12.98 (1H, s, NH); ^{13}C NMR (acetone-d_6, 100 MHz) δ: 10.9 (C–CH_3), 40.4 (N–CH_3), 122.4 (Ar–C), 123.7 (Ar–C), 124.8 (Ar–C), 125.1 (Ar–C), 125.6 (Ar–C), 125.8 (Ar–C), 126.0 (Ar–C), 128.3 (Ar–C), 128.6 (Ar–C), 128.8 (Ar–C), 129.0 (Ar–C), 129.5 (Ar–C), 130.0 (Ar–C), 130.2 (Ar–C), 131.5 (Ar–C), 132.5 (Ar–C), 133.8 (Ar–C), 134.0 (Ar–C), 134.9 (Ar–C), 139.8 (Ar–C), 141.8 (N=C, Ar–C), 154.0 (N=C–N, Ar–C); MS *m*/*z*: 485 (ES–), 487 (ES+); HR-MS (ES–) calcd for $C_{24}H_{18}N_6O_4S$, 486.1110; found, 485.1039.

*6.1.6.8. 2-[4-(5-Methoxy-1H-benzimidazol-2-yl)phenyl]-3,4-dimethyl-2,4-dihydropyrazolo[4,3-c][1,2]benzothiazine 5,5-dioxide (**6h**).* Light brown powder; Yield: 89%; FT-IR (KBr): 3019, 2973, 2929, 2912, 1731, 1600, 1506, 1475, 1375, 1232, 1160, 847, 773, 746 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 2.44 (3H, s, C–CH_3), 3.01 (3H, s, N–CH_3), 3.87 (3H, s, O–CH_3), 7.01–7.04 (1H, dd, J_1 = 2.0 Hz, J_2 = 8.8 Hz, Ar–*H*), 7.20 (1H, d, J = 2.0 Hz, Ar–*H*), 7.41 (1H, d, J = 8.4 Hz, Ar–*H*), 7.59 (1H, s, Ar–*H*), 7.65 (1H, d, J = 8.8 Hz, Ar–*H*), 7.73 (1H, t, J = 8.0 Hz, Ar–*H*), 7.86 (1H, t, J = 7.6 Hz, Ar–*H*), 7.97 (1H, d, J = 8.4 Hz, Ar–*H*), 8.09 (1H, d, J = 7.6 Hz, Ar–*H*), 8.25 (1H, d, J = 8.4 Hz, Ar–*H*), 8.36 (1H, d, J = 8.4 Hz, Ar–*H*), 12.97 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 10.4 (C–CH_3), 40.1 (N–CH_3), 55.7 (O–CH_3), 113.8 (Ar–C), 123.8 (Ar–C), 124.4 (Ar–C), 124.5 (Ar–C), 125.0 (Ar–C), 127.3 (Ar–C), 127.8 (Ar–C), 128.5 (Ar–C), 129.0 (Ar–C), 129.8 (Ar–C), 130.1 (Ar–C), 130.2 (Ar–C), 131.9 (Ar–C), 132.4 (Ar–C), 133.6 (Ar–C), 134.0 (Ar–C), 134.2 (Ar–C), 138.4 (Ar–C), 140.6 (Ar–C), 148.7 (N=C, Ar–C), 152.3 (N=C–N, Ar–C), 157.0 (Ar–C); MS *m*/*z*: 470 (ES–), 472 (ES+); HR-MS (ES–) calcd for $C_{25}H_{21}N_5O_3S$, 471.1365; found, 470.1303.

*6.1.6.9. 3,4-Dimethyl-2-[4-(9H-purin-8-yl)phenyl]-2,4-dihydropyrazolo[4,3-c][1,2] benzothiazine 5,5-dioxide (**6i**).* Brown powder; Yield: 83%; FT-IR (KBr): 3062, 2927, 2873, 1699, 1663, 1600, 1490, 1436, 1375, 1342, 1245, 1169, 1063, 849, 772, 746 cm^{-1}; ^{1}H NMR (acetone-d_6, 400 MHz) δ: 2.47 (3H, s, C–CH_3), 2.98 (3H, s, N–CH_3), 7.47 (1H, d, J = 2.4 Hz, Ar–*H*), 7.56–7.60 (1H, dt, J_1 = 1.2 Hz, J_2 = 7.6 Hz, J_3 = 15.6 Hz, Ar–*H*), 7.67–7.72 (1H, dt, J_1 = 1.2 Hz, J_2 = 7.6 Hz, J_3 = 18.8 Hz, Ar–*H*), 7.82 (1H, t, J = 8.4 Hz, Ar–*H*), 7.99 (1H, d, J = 7.6 Hz, Ar–*H*), 8.40 (2H, d, J = 8.8 Hz, Ar–*H*), 8.46 (1H, d, J = 8.8 Hz, Ar–*H*), 8.76 (1H, s, Ar–*H*), 8.95 (1H, s, Ar–*H*), 11.57 (1H, s, NH); ^{13}C NMR (acetone-d_6, 100 MHz) δ: 10.8 (C–CH_3), 40.4 (N–CH_3), 122.4 (Ar–C), 123.6 (Ar–C), 124.9 (Ar–C), 125.1 (Ar–C), 125.5 (Ar–C), 127.0 (Ar–C), 128.5 (Ar–C), 128.8 (Ar–C), 129.7 (Ar–C), 130.1 (Ar–C), 130.3 (Ar–C), 133.6 (Ar–C), 134.0 (Ar–C), 135.0 (Ar–C), 139.6 (Ar–C), 141.6 (N=C, pyr Ar–C), 147.8 (N=C, purine Ar–C), 151.8 (N–C=N, purine Ar–C), 155.8 (N–C=C, purine Ar–C), 157.9 (N=C–N, Ar–C); MS *m*/*z*: 442 (ES–), 444 (ES+); HR-MS (ES–) calcd for $C_{22}H_{17}N_7O_2S$, 443.1164; found, 442.1084.

*6.1.6.10. 2-[4-(1,3-Benzoxazol-2-yl)phenyl]-3,4-dimethyl-2,4-dihydropyrazolo[4,3-c][1,2]benzothiazine 5,5-dioxide (**6j**).* Yellowish powder; Yield: 87%; FT-IR (KBr): 3098, 3034, 2958, 1749, 1601, 1509, 1438, 1374, 1232, 1169, 1064, 843, 768, 747 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.45 (3H, s, C–CH_3), 3.06 (3H, s, N–CH_3), 6.85–6.88 (1H, dt, J_1 = 1.2 Hz, J_2 = 7.6 Hz, J_3 = 15.2 Hz, Ar–*H*), 6.97 (1H, d, J = 8.8 Hz, Ar–*H*), 7.27–7.30 (4H, dd, J_1 = 1.2 Hz, J_2 = 8.0 Hz, Ar–*H*), 7.50–7.55 (2H, m, Ar–*H*), 7.61 (1H, t, J = 8.0 Hz, Ar–*H*), 7.67 (1H, t, J = 8.4 Hz, Ar–*H*), 7.90 (1H, d, J = 8.8 Hz, Ar–*H*), 8.01 (1H, t, J = 8.4 Hz, Ar–*H*); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 10.6 (C–CH_3), 40.0 (N–CH_3), 115.3 (Ar–C), 115.9 (Ar–C), 120.2 (Ar–C), 124.2 (Ar–C), 124.8 (Ar–C), 124.9 (Ar–C), 125.0 (Ar–C), 125.1 (Ar–C), 125.8 (Ar–C), 126.7 (Ar–C), 128.7 (Ar–C), 128.8 (Ar–C), 129.2 (Ar–C), 129.4 (Ar–C), 129.5 (Ar–C), 129.7 (Ar–C), 130.8 (Ar–C), 132.9 (Ar–C), 133.0 (Ar–C), 135.1 (Ar–C), 141.3 (N=C, Ar–C), 155.3 (N=C–N, Ar–C); MS *m*/*z*: 443 (ES–), 445 (ES+); HR-MS (ES–) calcd for $C_{24}H_{18}N_4O_3S$, 442.1100; found, 443.1183.

*6.1.6.11. Ethyl 2-(4-(3,4-dimethyl-5,5-dioxidobenzo[e]pyrazolo[4,3-c][1,2] thiazin-2(4H)-yl)phenyl)-1H-benzo[d]imidazole-5-carboxylate (**6k**).*

2-[4-(5-Carboxy-1*H*-benzimidazol-2-yl)phenyl]-3,4-dimethyl-2,4-dihydropyrazolo[4,3-*c*][1,2]benzothiazine 5,5-dioxide (**6a**) (1.0 g) was dissolved in ethanol (20 mL) and a catalytic amount of conc. H_2SO_4 was added. The reaction mixture refluxed for 30–40 min and completion of the reaction was confirmed by TLC. The solvent was removed under vacuum. The product was dissolved in ice cold water and a pH 7–8 was adjusted by using 5% $NaHCO_3$. The formed precipitate was filtered, washed with water and dried. Off white powder; yield: 70%; FT-IR (KBr): 3380, 2990, 2937, 1722, 1630, 1605, 1506, 1439, 1369, 1296, 1170, 1001, 843, 767, 743 cm^{-1}; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.45 (3H, t, J = 7.2 Hz, C–CH_3), 2.60 (3H, s, C–CH_3), 3.01 (3H, s, N–CH_3), 4.46 (2H, q, J = 7.2 Hz, O–CH_2), 7.62 (1H, t, J = 9.2 Hz, Ar–*H*), 7.81 (1H, t, J = 7.6 Hz, Ar–*H*), 7.94 (1H, t, J = 7.6 Hz, Ar–*H*), 8.07–8.09 (4H, dd, J_1 = 8.0 Hz, J_2 = 20.4 Hz, Ar–*H*), 8.17 (1H, t, J = 8.4 Hz, Ar–*H*), 8.23 (1H, s, Ar–*H*), 8.51 (2H, t, J = 8.8 Hz, Ar–*H*), 12.98 (1H, s, NH); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 10.5 (C–CH_3), 14.2 (H_2C–CH_3), 40.0 (N–CH_3), 60.7 (O–CH_2), 114.6 (Ar–C), 122.4 (Ar–C), 124.5 (Ar–C), 124.7 (Ar–C), 125.0 (Ar–C), 125.3 (Ar–C), 125.8 (Ar–C), 127.1 (Ar–C), 128.4 (Ar–C), 128.9 (Ar–C), 129.5 (Ar–C), 130.0 (Ar–C), 130.2 (Ar–C), 130.5 (Ar–C), 131.5 (Ar–C), 132.5 (Ar–C), 133.6 (Ar–C), 134.0 (Ar–C), 134.5 (Ar–C), 139.8 (Ar–C), 141.9 (N=C, Ar–C), 150.4 (N=C–N, Ar–C), 162.2 (C=O); MS *m*/*z*: 512 (ES–), 514 (ES+); HR-MS (ES+) calcd for $C_{27}H_{23}N_5O_4S$, 513.1471; found, 514.1548 ($M + H^+$).

*6.1.6.12. 2-(4-(4-Methyl-5,5-dioxido-3-phenylbenzo[e]pyrazolo[4,3-c][1,2] thiazin-2(4H)-yl)phenyl)-1H-benzo[d]imidazole-5-carboxylic acid (**12a**).* Brown powder; Yield: 86%; FT-IR (KBr): 3650, 2944, 2920, 1648, 1490, 1437, 1382, 1289, 1094, 967, 845 cm^{-1}; ^{1}H NMR (acetone-d_6, 400 MHz) δ: 2.98 (3H, s, N–CH_3), 7.02 (1H, d, J = 7.6 Hz, Ar–*H*), 7.37 (1H, t, J = 7.6 Hz, Ar–*H*), 7.29–7.33 (2H, dd, J_1 = 6.0 Hz, J_2 = 9.2 Hz, Ar–*H*), 7.53–7.57 (2H, dd, J_1 = 6.4 Hz, J_2 = 12.8 Hz, Ar–*H*), 7.65 (1H, d, J = 6.8 Hz, Ar–*H*), 7.69 (1H, t, J = 8.8 Hz, Ar–*H*), 7.89–7.93 (2H, m, Ar–*H*), 7.95 (2H, d, J = 7.6 Hz, Ar–*H*), 8.01 (1H, d, J = 7.6 Hz, Ar–*H*), 8.08 (1H, d, J = 8.8 Hz, Ar–*H*), 8.24 (1H, t, J = 7.6 Hz, Ar–*H*), 8.33 (1H, d, J = 9.2 Hz, Ar–*H*), 12.83 (1H,s,OH), 13.01 (1H, s, NH); ^{13}C NMR (acetone-d_6, 100 MHz) δ: 40.3 (N–CH_3), 116.9 (Ar–C), 119.0 (Ar–C), 120.2 (Ar–C), 122.4 (Ar–C), 123.4 (Ar–C), 123.7 (Ar–C), 124.0 (Ar–C), 124.8 (Ar–C), 125.1 (Ar–C), 125.5 (Ar–C), 125.8 (Ar–C), 126.0 (Ar–C), 128.4 (Ar–C), 128.7 (Ar–C), 128.9 (Ar–C), 129.1 (Ar–C), 129.8 (Ar–C), 130.2 (Ar–C), 130.5 (Ar–C), 131.5 (Ar–C), 132.5 (Ar–C), 133.8 (Ar–C), 134.0 (Ar–C), 135.0 (Ar–C), 139.8 (Ar–C), 141.9 (Ar–C), 143.7 (N=C, Ar–C), 152.9 (N=C–N, Ar–C), 168.2 (C=O); MS *m*/*z*: 546 (ES–), 548 (ES+); HR-MS (ES–) calcd for $C_{30}H_{21}N_5O_4S$, 547.1314; found 546.1249.

*6.1.6.13. 2-[4-(5-Chloro-1H-benzimidazol-2-yl)phenyl]-4-methyl-3-phenyl-2,4-dihydropyrazolo[4,3-c][1,2]benzothiazine 5,5-dioxide (**12b**).* Brown powder; Yield: 86%; FT-IR (KBr): 3113, 3075, 2953, 1656, 1601, 1490, 1436, 1369, 1234, 1170, 1059, 847, 771 cm^{-1}; ^{1}H NMR (acetone-d_6, 400 MHz) δ: 2.98 (3H, s, N–CH_3), 7.08–7.10 (1H, d, J = 2.0 Hz, Ar–*H*), 7.09 (1H, m, Ar–*H*), 7.13 (1H, q, J = 2.4 Hz, Ar–*H*), 7.18 (1H, d, J = 7.2 Hz, Ar–*H*), 7.33 (1H, t, J = 7.2 Hz, Ar–*H*), 7.37 (1H, s, Ar–*H*), 7.43–7.46 (2H, dd, J_1 = 8.0 Hz, J_2 = 15.6 Hz, Ar–*H*), 7.49 (1H, q, J = 6.8 Hz, Ar–*H*), 7.56–7.58 (1H, dt, J_1 = 1.2 Hz, J_2 = 5.2 Hz, J_3 = 8.0 Hz, Ar–*H*), 7.59–7.60 (1H, dd, J_1 = 1.2 Hz, J_2 = 4.4 Hz, Ar–*H*), 7.70 (1H, q, J = 8.4 Hz, Ar–*H*), 7.83 (1H, t,

J = 8.0 Hz, Ar–*H*), 7.89–7.91 (1H, dd, J_1 = 1.2 Hz, J_2 = 7.6 Hz, Ar–*H*), 7.96 (1H, d, J = 8.0 Hz, Ar–*H*), 8.00–8.26 (1H, dd, J_1 = 1.2 Hz, J_2 = 8.4 Hz, Ar–*H*), 8.07 (1H, d, J = 7.6 Hz, Ar–*H*), 8.11 (1H, d, J = 8.8 Hz, Ar–*H*), 8.30 (2H, q, J = 8.4 Hz, Ar–*H*) 13.13 (1H,s,NH); ^{13}C NMR (acetone-d_6, 100 MHz) δ: 40.3 (N–CH_3), 116.9 (Ar–C), 118.9 (Ar–C), 123.5 (Ar–C), 123.7 (Ar–C), 124.1 (Ar–C), 124.5 (Ar–C), 124.8 (Ar–C), 124.8 (Ar–C), 125.2 (Ar–C), 125.5 (Ar–C), 125.8 (Ar–C), 126.0 (Ar–C), 127.4 (Ar–C), 128.2 (Ar–C), 128.7 (Ar–C), 128.9 (Ar–C), 129.1 (Ar–C), 129.9 (Ar–C), 130.0 (Ar–C), 130.5 (Ar–C), 131.0 (Ar–C), 131.6 (Ar–C), 133.6 (Ar–C), 134.1 (Ar–C), 134.9 (Ar–C), 139.8 (Ar–C), 141.9 (N=C, Ar–C), 152.6 (N=C–N, Ar–C); MS *m*/*z*: 536 (ES–), 538 (ES+), HR-MS (ES–) cacld for $C_{29}H_{20}ClN_5O_2S$, 537.1026; found, 536.0950.

*6.1.6.14. 2-[4-(5-Bromo-1H-benzimidazol-2-yl)phenyl]-4-methyl-3-phenyl-2,4-dihydropyrazolo[4,3-c][1,2]benzothiazine 5,5-dioxide (**12c**).* Brown powder; Yield: 84%; FT-IR (KBr): 3403, 2926, 1652, 1491, 1462, 1386, 1344, 1253, 1131, 1086, 913, 848, 747 cm^{-1}; ^{1}H NMR (acetone-d_6, 400 MHz) δ: 2.97 (3H, s, N–CH_3), 7.18–7.26 (2H, m, Ar–*H*), 7.34 (1H, d, J = 6.4 Hz, Ar–*H*), 7.44–7.47 (2H, dd, J_1 = 3.2 Hz, J_2 = 8.4 Hz, Ar–*H*), 7.68–7.73 (4H, dd, J_1 = 8.4 Hz, J_2 = 12.8 Hz, Ar–*H*), 7.84 (1H, s, Ar–*H*), 7.97–8.03 (2H, dd, J_1 = 7.6 Hz, J_2 = 18.0 Hz, Ar–*H*), 8.07–8.13 (2H, dd, J_1 = 7.2 Hz, J_2 = 16.8 Hz, Ar–*H*), 8.31 (2H, q, J = 8.4 Hz, Ar–*H*) 13.14 (1H, s, NH); ^{13}C NMR (acetone-d_6, 100 MHz) δ: 40.3 (N–CH_3), 117.7 (Ar–C), 123.5, 123.7, 124.1 (Ar–C), 124.5 (Ar–C), 124.8 (Ar–C), 125.0 (Ar–C), 125.3 (Ar–C), 125.5 (Ar–C), 125.8 (Ar–C), 126.1 (Ar–C), 126.3 (Ar–C), 126.5 (Ar–C), 127.4 (Ar–C), 128.3 (Ar–C), 128.5 (Ar–C), 128.9 (Ar–C), 129.9 (Ar–C), 130.0 (Ar–C), 130.5 (Ar–C), 131.0 (Ar–C), 131.6 (Ar–C), 133.6 (Ar–C), 134.0 (Ar–C), 134.9 (Ar–C), 139.8 (Ar–C), 141.8 (N=C, Ar–C), 152.8 (N=C–N, Ar–C); MS *m*/*z*: 580 (ES–), 582 (ES+) (M + 2); HR-MS (ES–) calcd for $C_{29}H_{20}BrN_5O_2S$, 581.0521; found, 580.0452.

*6.1.6.15. 2-[4-(1H-Benzimidazol-2-yl)phenyl]-4-methyl-3-phenyl-2,4-dihydropyrazolo[4,3-c][1,2]benzothiazine 5,5-dioxide (**12d**).* Brown powder; Yield: 85%; FT-IR (KBr): 2998, 2935, 1661, 1598, 1496, 1437, 1347, 1276, 1171, 1062, 887, 746 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.89 (3H, s, N–CH_3), 7.03 (1H, d, J = 8.0 Hz, Ar–*H*), 7.13 (2H, q, J = 3.2 Hz, Ar–*H*), 7.18 (1H, d, J = 7.2 Hz, Ar–*H*), 7.26–7.33 (4H, dd, J_1 = 8.0 Hz, J_2 = 20.4 Hz, Ar–*H*), 7.38 (1H, t, J = 7.2 Hz, Ar–*H*), 7.46 (2H, d, J = 8.4 Hz, Ar–*H*), 7.50 (1H, t, J = 8.0 Hz, Ar–*H*), 7.90 (2H, t, J = 7.6 Hz, Ar–*H*), 8.11 (2H, d, J = 8.4 Hz, Ar–*H*), 8.16 (1H, d, J = 8.4 Hz, Ar–*H*) 13.10 (1H, s, NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 40.0 (N–CH_3), 115.4 (Ar–C), 115.9 (Ar–C), 123.1 (Ar–C), 124.2 (Ar–C), 124.4 (Ar–C), 124.7 (Ar–C), 124.9 (Ar–C), 125.2 (Ar–C), 125.3 (Ar–C), 125.4 (Ar–C), 126.1 (Ar–C), 126.8 (Ar–C), 127.4 (Ar–C), 127.6 (Ar–C), 128.0 (Ar–C), 128.9 (Ar–C), 129.1 (Ar–C), 129.2 (Ar–C), 129.5 (Ar–C), 129.8 (Ar–C), 130.4 (Ar–C), 132.4 (Ar–C), 132.9 (Ar–C), 135.9 (Ar–C), 139.5 (Ar–C), 140.6 (N=C, Ar–C), 150.6 (Ar–C), 152.7 (N=C–N, Ar–C); MS *m*/*z*: 502 (ES–), 504 (ES+); HR-MS (ES–) calcd for $C_{29}H_{21}N_5O_2S$, 503.1416; found, 502.1357.

*6.1.6.16. 2-[4-(5-Methyl-1H-benzimidazol-2-yl)phenyl]-4-methyl-3-phenyl-2,4-dihydropyrazolo[4,3-c][1,2]benzothiazine 5,5-dioxide (**12e**).* Off white powder; Yield: 82%; FT-IR (KBr): 3084, 2982, 1736, 1663, 1597, 1437, 1347, 1229, 1170, 1062, 848, 772 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.81 (3H, s, C–CH_3), 2.89 (3H, s, N–CH_3), 7.02 (1H, q, J = 8.4 Hz, Ar–*H*), 7.26–7.34 (2H, m, Ar–*H*), 7.41 (1H, t, J = 8.0 Hz, Ar–*H*), 7.47 (1H, t, J = 8.0 Hz, Ar–*H*), 7.52 (1H, t, J = 8.4 Hz, Ar–*H*), 7.56–7.59 (4H, dd, J_1 = 1.2 Hz, J_2 = 8.8 Hz, Ar–*H*), 7.60–7.64 (1H, dt, J_1 = 1.2 Hz, J_2 = 7.6 Hz, J_3 = 16.4 Hz, Ar–*H*), 7.90 (1H, t, J = 8.0 Hz, Ar–*H*), 7.94 (2H, d, J = 7.2 Hz, Ar–*H*), 7.99 (1H, t, J = 8.4 Hz, Ar–*H*), 8.08 (1H, d, J = 8.4 Hz, Ar–*H*), 13.05 (1H, s, NH). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 29.7 (C–CH_3), 40.4 (N–CH_3), 117.6 (Ar–C), 123.0 (Ar–C), 123.3 (Ar–C), 124.2 (Ar–C), 124.3 (Ar–C), 124.7 (Ar–C), 124.9 (Ar–C), 125.3 (Ar–C), 125.3 (Ar–C), 125.6 (Ar–C), 126.0 (Ar–C), 126.8 (Ar–C), 127.2 (Ar–C), 127.8 (Ar–C), 128.0 (Ar–C), 129.0 (Ar–C), 129.1 (Ar–C), 129.2 (Ar–C), 129.6 (Ar–C), 129.6 (Ar–C), 130.4 (Ar–C), 132.3 (Ar–C), 132.9 (Ar–C), 135.7 (Ar–C), 139.4 (Ar–C), 140.5 (Ar–C), 145.6 (N=C, Ar–C), 152.5 (N=C–N, Ar–C); MS *m*/*z*: 516 (ES–), 518 (ES+); HR-MS (ES–) calcd for $C_{30}H_{23}N_5O_2S$, 517.1572; found, 516.1495.

*6.1.6.17. 2-[4-(5,6-Dimethyl-1H-benzimidazol-2-yl)phenyl]-4-methyl-3-phenyl-2,4-dihydropyrazolo[4,3-c][1,2]benzothiazine 5,5-dioxide (**12f**).* Dirty yellow powder; Yield: 81%; FT-IR (KBr): 3067, 2980, 1737, 1631, 1508, 1438, 1350, 1171, 1062, 849, 773 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.81 (6H, s, C–CH_3), 2.89 (3H, s, N–CH_3), 7.30–7.33 (2H, m, Ar–*H*), 7.41 (2H, d, J = 6.3 Hz, Ar–*H*), 7.50 (3H, t, J = 6.0 Hz, Ar–*H*), 7.82 (2H, t, J = 6.0 Hz, Ar–*H*), 7.89 (1H, t, J = 9.0 Hz, Ar–*H*), 8.01–8.04 (2H, m, Ar–*H*), 8.15 (2H, d, J = 8.4 Hz, Ar–*H*), 13.06 (1H, s, NH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 29.7 (=C–CH_3), 31.5 (=C–CH_3), 40.4 (N–CH_3), 116.8 (Ar–C), 123.1 (Ar–C), 123.1 (Ar–C), 124.1 (Ar–C), 124.4 (Ar–C), 124.7 (Ar–C), 125.0 (Ar–C), 125.2 (Ar–C), 125.4 (Ar–C), 125.7 (Ar–C), 126.0 (Ar–C), 126.8 (Ar–C), 127.4 (Ar–C), 127.6 (Ar–C), 127.8 (Ar–C), 128.9 (Ar–C), 129.1 (Ar–C), 129.2 (Ar–C), 129.5 (Ar–C), 129.7 (Ar–C), 130.4 (Ar–C), 132.2 (Ar–C), 132.8 (Ar–C), 135.8 (Ar–C), 139.7 (Ar–C), 141.6 (N=C, Ar–C), 150.6 (Ar–C), 151.7 (N=C–N, Ar–C); MS *m*/*z*: 530 (ES–), 532 (ES+); HR-MS (ES–) calcd for $C_{31}H_{25}N_5O_2S$, 531.1729; found, 530.1631.

*6.1.6.18. 2-[4-(5-Nitro-1H-benzimidazol-2-yl)phenyl]-4-methyl-3-phenyl-2,4-dihydropyrazolo[4,3-c][1,2]benzothiazine 5,5-dioxide (**12g**).* Light brown powder; Yield: 85%; FT-IR (KBr): 3456, 3093, 1748, 1647, 1509, 1438, 1340, 1248, 1171, 1062, 850, 772 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.92 (3H, s, N–CH_3), 7.09 (1H, d, J = 8.0 Hz, Ar–*H*), 7.30 (1H, t, J = 3.2 Hz, Ar–*H*), 7.34–7.41 (2H, dt, J_1 = 2.0 Hz, J_2 = 8.0 Hz, J_3 = 16.4 Hz, Ar–*H*), 7.48 (2H, q, J = 8.0 Hz, Ar–*H*), 7.55–7.60 (4H, dd, J_1 = 8.0 Hz, J_2 = 16.4 Hz, Ar–*H*), 7.90 (1H, t, J = 8.8 Hz, Ar–*H*), 7.94 (1H, t, J = 8.4 Hz, Ar–*H*), 8.01 (2H, d, J = 8.0 Hz, Ar–*H*), 8.09 (1H, t, J = 11.2 Hz, Ar–*H*), 8.45 (1H, s, Ar–*H*), 13.13 (1H, s, NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 40.3 (N–CH_3),120.0 (Ar–C), 123.5 (Ar–C), 123.7 (Ar–C), 124.2 (Ar–C), 124.6 (Ar–C), 124.8 (Ar–C), 124.9 (Ar–C), 125.1 (Ar–C), 125.1 (Ar–C), 125.2 (Ar–C), 125.5 (Ar–C), 126.7 (Ar–C), 127.6 (Ar–C), 127.9 (Ar–C), 128.3 (Ar–C), 128.5 (Ar–C), 129.0 (Ar–C), 129.2 (Ar–C), 129.5 (Ar–C), 129.8 (Ar–C), 131.0 (Ar–C), 131.6 (Ar–C), 133.1 (Ar–C), 134.2 (Ar–C), 135.0 (Ar–C), 139.8 (Ar–C), 141.9 (N=C, Ar–C), 152.9 (N=C–N, Ar–C); MS *m*/*z*: 547 (ES–), 549 (ES+); HR-MS (ES–) calcd for $C_{29}H_{20}N_6O_4S$, 548.1267; found, 547.1182.

*6.1.6.19. 2-[4-(5-Methoxy-1H-benzimidazol-2-yl)phenyl]-4-methyl-3 phenyl-2,4-dihydropyrazolo[4,3-c][1,2]benzothiazine 5,5-dioxide (**12h**).* Brown powder; Yield: 84%; FT-IR (KBr): 3100, 3089, 1662, 1478, 1369, 1349, 1232, 1160, 1063, 1006, 970, 848, 774, 748 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 2.91 (3H, s, N–CH_3), 3.70 (3H, s, O–CH_3), 6.80 (1H, t, J = 9.0 Hz, Ar–*H*), 7.03 (1H, d, J = 6.0 Hz, Ar–*H*), 7.27 (1H, s, Ar–*H*), 7.33 (2H, t, J = 5.7 Hz, Ar–*H*), 7.39 (2H, t, J = 5.7 Hz, Ar–*H*), 7.43–7.50 (4H, m, Ar–*H*), 7.90 (1H, t, J = 5.7 Hz, Ar–*H*), 7.96 (2H, q, J = 5.1 Hz, Ar–*H*), 8.04 (1H, t, J = 5.7 Hz, Ar–*H*), 8.13 (1H, d, J = 6.0 Hz, Ar–*H*), 13.11 (1H, s, NH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 40.4 (N–CH_3), 55.8 (O–CH_3), 123.0 (Ar–C), 123.5 (Ar–C), 123.7 (Ar–C), 124.2 (Ar–C), 124.6 (Ar–C), 124.8 (Ar–C), 125.0 (Ar–C), 125.0 (Ar–C), 125.1 (Ar–C), 125.2 (Ar–C), 125.5 (Ar–C), 126.8 (Ar–C), 127.8 (Ar–C), 127.9 (Ar–C), 128.2 (Ar–C), 128.4 (Ar–C), 129.0 (Ar–C), 129.1 (Ar–C), 129.3 (Ar–C), 129.8 (Ar–C), 130.6 (Ar–C), 131.4 (Ar–C), 133.0 (Ar–C), 134.4 (Ar–C), 134.8 (Ar–C), 137.8 (Ar–C), 141.9 (N=C, Ar–C), 151.7 (N=C–N, Ar–C); MS *m*/*z*: 532

(ES−), 534 (ES+); HR-MS (ES−) calcd for $C_{30}H_{23}N_5O_3S$, 533.1522; found, 532.1440.

*6.1.6.20. 4-Methyl-3-phenyl-2-[4-(9H-purin-8-yl)phenyl]-2,4-dihydropyrazolo[4,3c][1,2]benzothiazine 5,5-dioxide (**12i**).* Yellowish powder; Yield: 85%; FT-IR (KBr): 3348, 3069, 2854, 1660, 1614, 1487, 1436, 1369, 1345, 1233, 1171, 1062, 1006, 969, 851, 773, 747 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ: 2.91 (3H, s, N–CH_3), 7.15 (1H, d, J = 5.7 Hz, Ar–*H*), 7.36 (1H, d, J = 5.4 Hz, Ar–*H*), 7.42 (1H, t, J = 5.4 Hz, Ar–*H*), 7.48–7.55 (2H, m, Ar–*H*), 7.65 (1H, t, J = 6.6 Hz, Ar–*H*), 7.76 (1H, d, J = 6.0 Hz, Ar–*H*), 7.90 (1H, t, J = 5.4 Hz, Ar–*H*), 7.97 (2H, d, J = 5.4 Hz, Ar–*H*), 8.02 (1H, t, J = 5.7 Hz, Ar–*H*), 8.16 (1H, d, J = 6.3 Hz, Ar–*H*), 8.35 (1H, d, J = 6.3 Hz, Ar–*H*), 8.98 (1H, s, =N–C–*H*), 9.15 (1H, s, =N–C–*H*), 11.76 (1H, s, NH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 40.0 (N–CH_3), 123.7 (Ar–C), 124.3 (Ar–C), 124.4 (Ar–C), 124.6 (Ar–C), 124.8 (Ar–C), 125.0 (Ar–C), 125.4 (Ar–C), 126.8 (Ar–C), 127.6 (Ar–C), 127.9 (Ar–C), 128.2 (Ar–C), 128.5 (Ar–C), 128.8 (Ar–C), 129.0 (Ar–C), 129.2 (Ar–C), 129.4 (Ar–C), 129.8 (Ar–C), 130.5 (Ar–C), 132.4 (Ar–C), 133.0 (Ar–C), 134.2 (Ar–C), 135.0 (Ar–C), 139.8 (Ar–C), 142.1 (N=C, Ar–C), 145.5 (N=C–N, Ar–C), 155.5 (N=C, Ar–C); MS m/z: 504 (ES−), 506 (ES+); HR-MS (ES−) calcd for $C_{27}H_{19}N_7O_2S$, 505.1321; found, 504.1246.

*6.1.6.21. 2-[4-(1,3-Benzoxazol-2-yl)phenyl]-4-methyl-3-phenyl-2,4-dihydropyrazolo[4,3-c][1,2]benzothiazine 5,5-dioxide (**12j**).* Brown powder; Yield: 83%; FT-IR (KBr): 3085, 2963, 1651, 1596, 1490, 1437, 1371, 1236, 1170, 845, 772 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ: 2.89 (3H, s, N–CH_3), 7.05 (1H, d, J = 8.0 Hz, Ar–*H*), 7.15 (2H, q, J = 6.8 Hz, Ar–*H*), 7.21 (1H, d, J = 7.2 Hz, Ar–*H*), 7.27–7.32 (4H, dd, J_1 = 4.8 Hz, J_2 = 8.4 Hz, Ar–*H*), 7.38 (1H, t, J = 7.2 Hz, Ar–*H*), 7.44 (2H, d, J = 8.4 Hz, Ar–*H*), 7.51 (1H, t, J = 8.0 Hz, Ar–*H*), 7.90 (2H, t, J = 7.6 Hz, Ar–*H*), 8.11 (2H, d, J = 8.0 Hz, Ar–*H*), 8.15 (1H, d, J = 8.0 Hz, Ar–*H*); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 40.3 (N–CH_3), 123.4 (Ar–C), 123.7 (Ar–C), 124.2 (Ar–C), 124.5 (Ar–C), 124.7 (Ar–C), 124.9 (Ar–C), 125.0 (Ar–C), 125.1 (Ar–C), 125.2 (Ar–C), 125.5 (Ar–C), 126.7 (Ar–C), 127.6 (Ar–C), 127.9 (Ar–C), 128.2 (Ar–C), 128.5 (Ar–C), 128.9 (Ar–C), 129.1 (Ar–C), 129.4 (Ar–C), 129.8 (Ar–C), 130.9 (Ar–C), 131.5 (Ar–C), 133.1 (Ar–C), 134.2 (Ar–C), 134.7 (Ar–C), 139.7 (Ar–C), 141.8 (N=C, Ar–C), 150.9 (Ar–C), 151.8 (N=C–O, Ar–C); MS m/z: 503 (ES−); HR-MS (ES−) calcd for $C_{29}H_{20}N_4O_3S$, 504.1256; found, 503.0575.

*6.1.6.22. Ethyl 2-(4-(4-methyl-5,5-dioxido-3-phenylbenzo[e]pyrazolo[4,3-c][1,2]thiazin-2(4H)-yl)phenyl)-1H-benzo[d]imidazole-5-carboxylate (**12k**).*
2-(4-(4-Methyl-5,5-dioxido-3-phenylbenzo[*e*]pyrazolo[4,3-*c*][1,2]thiazin-2(4*H*)-yl)phenyl)-1*H*-benzo[*d*]imidazole-5-carboxylic acid (**12a**) (1.0 g) was dissolved in ethanol (20 mL) and a catalytic amount of conc. H_2SO_4 was added. The reaction mixture was allowed to reflux for 30–40 min. Completion of the reaction was confirmed by TLC. The solvent was removed under vacuum. The resulting residue was dissolved in ice cold water and a pH of 7–8 was adjusted by using 5% $NaHCO_3$. The precipitate formed was filtered, excessively washed with water and dried. Off white powder; yield: 71%; FT-IR (KBr): 3060, 2928, 2858, 1713, 1608, 1505, 1453, 1350, 1282, 1184, 1061, 848, 771 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ: 1.34 (3H, t, J = 5.7 Hz, C–CH_3), 2.97 (3H, s, N–CH_3), 4.22 (2H, q, J = 5.7 Hz, O–CH_2), 7.03 (1H, d, J = 5.7 Hz, Ar–*H*), 7.36 (1H, t, J = 5.7 Hz, Ar–*H*), 7.27–7.32 (2H, dd, J_1 = 4.5 Hz, J_2 = 10.2 Hz, Ar–*H*), 7.55–7.60 (2H, dd, J_1 = 4.2 Hz, J_2 = 10.8 Hz, Ar–*H*), 7.63 (1H, d, J = 5.1 Hz, Ar–*H*), 7.69 (1H, t, J = 6.6 Hz, Ar–*H*), 7.87–7.91 (3H, m, Ar–*H*), 7.94 (1H, d, J = 5.4 Hz, Ar–*H*), 8.02 (1H, d, J = 5.7 Hz, Ar–*H*), 8.08 (1H, d, J = 6.6 Hz, Ar–*H*), 8.26 (1H, t, J = 5.7 Hz, Ar–*H*), 8.32 (1H, d, J = 6.9 Hz, Ar–*H*), 8.49 (1H, s, NH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 29.7 (CH_3), 40.4 (N–CH_3), 55.0 (O–CH_2), 114.6 (Ar–C), 116.8, 123.8 (Ar–C), 124.2 (Ar–C), 124.3 (Ar–C), 124.4 (Ar–C), 124.5 (Ar–C), 124.8 (Ar–C), 125.2 (Ar–C), 125.4 (Ar–C), 125.6 (Ar–C), 126.2 (Ar–C), 126.8 (Ar–C), 127.6 (Ar–C), 127.9 (Ar–C), 128.3 (Ar–C), 128.8 (Ar–C), 129.0 (Ar–C), 129.2 (Ar–C), 129.3 (Ar–C), 129.9 (Ar–C), 131.4 (Ar–C), 133.1 (Ar–C), 134.9 (Ar–C), 135.2 (Ar–C), 139.6 (Ar–C), 141.5 (N=C, Ar–C), 151.6 (N=C–N, Ar–C), 162.8 (C=O); MS m/z: 574 (ES−), 576 (ES+); HR-MS (-ES) calcd for $C_{32}H_{25}N_5O_4S$, 575.1627; found, 574.1542.

6.2. Determination of AChE and BuChE inhibitory activity

Ellman's method was used to evaluate the inhibitory activities of newly synthesized compounds against EeAChE and equine BuChE [40]. The inhibitors studied were first dissolved in DMSO (final concentration of DMSO was 1% in the assay) and assayed at a 0.5 mM final concentration for initial screening. The compounds exhibiting more than 50% inhibition were further tested by making eight to ten serial dilutions in assay buffer (50 mM Tris–HCl, 0.1 M NaCl and 0.02 M $MgCl_2$, pH 8.0). Composition of the reaction mixture was: 20 μL assay buffer, 10 μL of test compound and 10 μL of enzymes (0.5 and 3.4 U/mg of EeAChE or equine BuChE, respectively). A 10 min-pre-incubation of the reaction mixture at 25 °C was carried out. At the end of the pre-incubation period, 10 μL of 1 mM acetylthiocholine iodide or butyrylthiocholine chloride were added to the respective EeAChE or equine BuChE enzyme solution to start the enzymatic reactions. The mixtures were incubated for 15 min at 25 °C. The change in the absorbance was measured at 405 nm to determine the amount of the product using a microplate reader (Bio-Tek ELx800). Neostigmine and donepezil were used as standard inhibitors. Assays were performed with a blank containing all of the components except enzyme or substrate in order to account for a non-enzymatic reaction. Each concentration was analysed in triplicate. Enzyme dilution buffer consisted of 50 mM Tris–HCl buffer containing 0.1% (w/v) bovine serum albumin (BSA) and pH 8.0. The linear regression parameters were determined for each curve and the IC_{50} values were measured. The computer program GraphPad Prism 5.0 (San Diego, CA, USA) was used to analyse the data.

6.3. Docking

The X-ray structures of human AChE (PDB ID 4BDT) [41] bound to huprine W and of human BuChE (PDB ID 4BDS) [41] bound to tacrine were obtained from the RCSB Protein Data Bank [42]. AutoDock 4.2 [43] was used for flexible ligand docking into the template X-ray structures. The side chain of residue Tyr337 in the active site of AChE was also treated flexibly during docking while all other residues were fixed in their crystallographic conformation. For BuChE, where residue Ala328 replaces Tyr337 in AChE, the entire active site was kept rigid. Prior to the calculations, crystallographic water and ligand molecules were removed from the template structures and hydrogen atoms were added using the Molecular Operating Environment (MOE 2012.10) [44]. AutoDock Tools [43] was used to add atomic partial charges. Selected hybrid molecules of pyrazolobenothiazines and benzimidazoles were docked into the active sites of AChE and BuChE using the Lamarckian Genetic Algorithm in AutoDock 4.2. High-scoring docking poses were selected as putative binding modes on the basis of visual inspection.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.03.035.

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