Journal of the Iranian Chemical Society (2022) 19:4457–4471
https://doi.org/10.1007/s13738-022-02614-8

ORIGINAL PAPER

New bis(pyrazolo[3,4-*b*]pyridines) and bis(thieno[2,3-*b*]pyridines) as potential acetylcholinesterase inhibitors: synthesis, in vitro and SwissADME prediction study

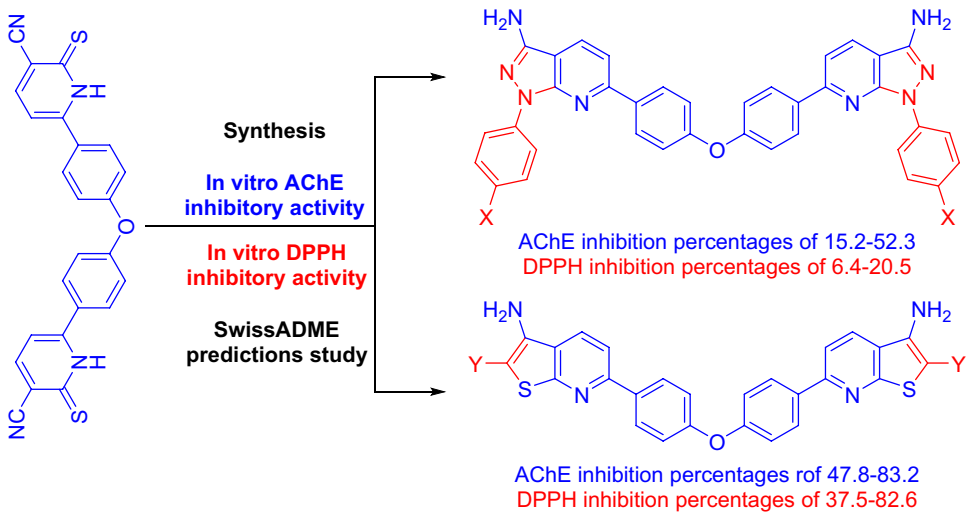
Ahmed A. M. Ahmed[1,2] · **Ahmed E. M. Mekky**[1] · **Sherif M. H. Sanad**[1]

Received: 24 January 2022 / Accepted: 15 June 2022 / Published online: 8 July 2022

Abstract

The bis(pyridine-2(1*H*)-thione) was prepared and taken as a key synthon of this study. The target bis(pyrazolo[3,4-*b*]pyridines) was prepared, in good yields, by the reaction of bis(pyridine-2(1*H*)-thione) with appropriate hydrazonyl chlorides to yield bis(hydrazonothioates) followed by their heating in ethanolic sodium ethoxide solution. Additionally, bis(pyridine-2(1*H*)-thione) reacted with different α-halogenated reagents to afford a new series of bis(thieno[2,3-*b*]pyridines), in good to excellent yields. In general, the tested series of bis(thieno[2,3-*b*]pyridines) demonstrated greater acetylcholinesterase inhibitory activity as well as DPPH antioxidant activity than the other series of (pyrazolo[3,4-*b*]pyridines). At a concentration of 100 µM, bis(thieno[2,3-*b*]pyridine-2-carbonitrile) showed the best acetylcholinesterase inhibitory activity with inhibition percentage of 83.2. In addition, the previous hybrid had the highest DPPH antioxidant activity, with an inhibition percentage of 82.6 when tested at a concentration of 25 µg/mL. Furthermore, SwissADME was used to predict the physicochemical properties, lipophilicity, and drug likeness of the new products.

Graphical abstract



Keywords DPPH antioxidant activity · Potential acetylcholinesterase inhibitors · Pyrazolo[3,4-*b*]pyridines · Thieno[2,3-*b*]pyridines · SwissADME predictions

✉ Ahmed E. M. Mekky
taher2211@cu.edu.eg

Extended author information available on the last page of the article

Introduction

Alzheimer's disease (AD) is the leading cause of dementia [1, 2]. A major symptom of the previous disease is a loss of intellectual and social abilities severe enough to interfere with everyday activities [3, 4]. The primary signs of AD are memory loss associated with a gradual reduction in cognitive functions such as intellectual abilities, as well as personality and behavioral changes [5]. The disease progresses from cognitive impairment to complete loss of mental capabilities, resulting in total reliance on others for everyday activities [6]. Brain tissue samples examined from patients suffering from AD demonstrated progressive accumulation of β-amyloid peptides (Aβ) in the form of senile plaques, as well as τ-protein aggregation into neurofibrillary tangles, resulting in neuron shrinkage and death [7, 8]. Oxidative stress and inflammation have also been linked to the incidence and development of AD [9, 10].

The cholinergic hypothesis has been proposed as the most leading theory explaining the etiology of AD by continued research aimed to identify the mechanism of AD progression. The finding of progressive loss of cholinergic neurons in areas of the brain involved in cognitive functions in AD patients has provided strong support for this hypothesis [11]. The previous hypothesis targets the serine hydrolase acetylcholinesterase enzyme (AChE), which is among the most valuable targeted therapies for mainly symptomatic AD [12, 13]. Moreover, numerous acetylcholinesterase inhibitors, such as tacrine, donepezil, galantamine, and rivastigmine, have been shown to halt the progression of AD and temporarily improve patients cognitive functions (see Fig. 1) [14–16]. Due to the limited number of AChE inhibitors presently available for the AD therapy, the search for new and potent inhibitors is a major focus of current research.

Both pyrazolo[3,4-*b*]pyridines and thieno[2,3-*b*]pyridines are a well-known class of heterocyclic derivatives due to their diverse biological properties. Several publications demonstrated the efficacy of the previous scaffolds as potent inhibitors of AChE [17–20]. Additionally, pyrazolo[3,4-*b*] pyridines have been shown to inhibit different cyclin-dependent kinases [21]. They can also act as antileukemic [22], anti-biofilm [23], antimicrobial [24], antiviral [25], antileishmanial [26], anticancer [27, 28], antimalarial [29], cardiovascular [30], antifungal [31], and anti-inflammatory [32]. Furthermore, thieno[2,3-*b*]pyridines have significant pharmacological activities as antimicrobial [33], antiviral [34], anti-inflammatory [35], and antidiabetic [36]. They are also effective inhibitors of DNA gyrase [37], and COX-2 enzymes [38]. Some potent biologically active pyrazolo[3,4-*b*]pyridines, and thieno[2,3-*b*]pyridines are presented in Fig. 2. [38–40]

SwissADME is a free web tool for assessing the pharmacokinetics, drug-likeness, and medicinal chemistry friendliness of hybrids under consideration. Accessing http://www.swissadme.ch in a web browser displays the SwissADME submission page, where molecules to be estimated can be entered [41].

Based on the foregoing findings and in the context of our continued effort to prepare potential AChE inhibitor [42–45], we aimed herein to prepare new bis(pyrazolo[3,4-*b*] pyridines) as well as bis(thieno[2,3-*b*]pyridines). Next, Ellman method was conducted to examine inhibitory capability of the target molecules. Furthermore, DPPH antioxidant activity, as well as SwissADME predictions of the physicochemical properties, and drug likeness of the new molecules were screened.

Results and discussion

Chemistry

Bis(2-thioxo-1,2-dihydropyridine-3-carbonitrile) **5** was first prepared and used as a key building block in this study. For this purpose, bis(acetyl) **1** [46] reacted with dimethylformamide-dimethylacetal (DMF-DMA) **2** in toluene at reflux for 18 h to give the corresponding bis(enaminone) **3** in 72% yield [47, 48]. The previous compound reacted with two equivalents of 2-cyanothioacetamide **4** in the presence of triethylamine (TEA) in dioxane at reflux for 6 h to give the synthon **5** in 63% yield (see Scheme 1 and Experimental section).

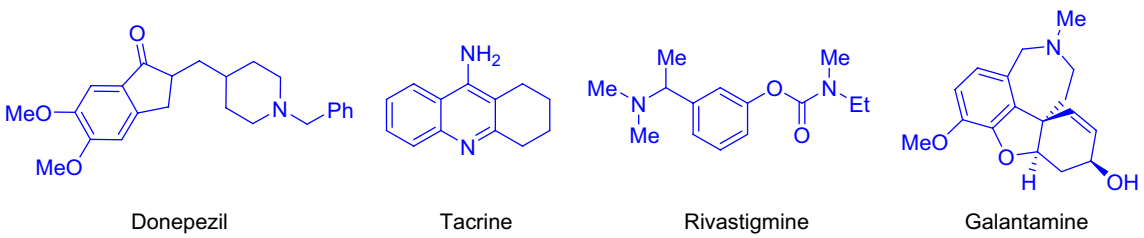


Fig. 1 Structure of some AChE inhibitors currently available for the treatment of AD

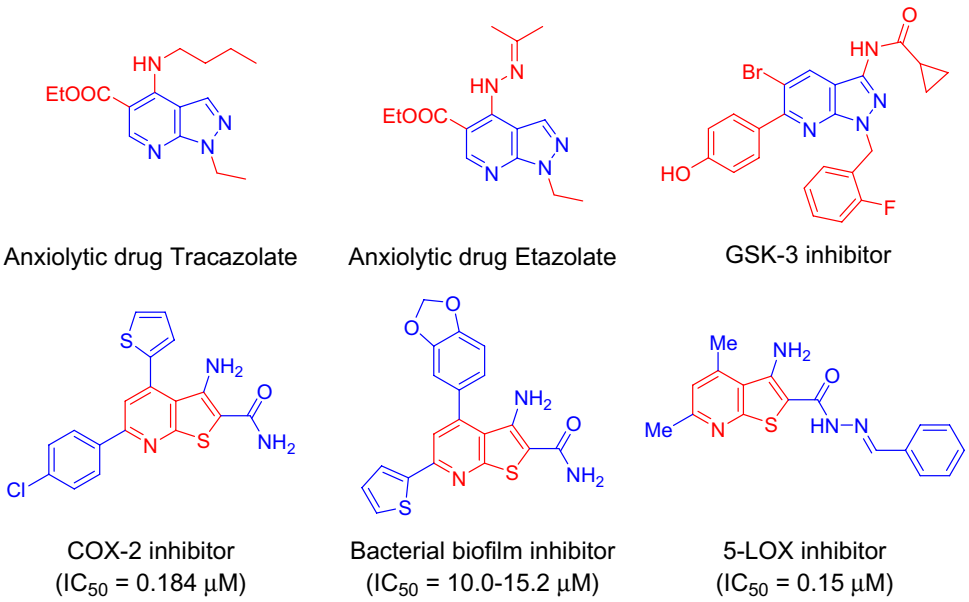
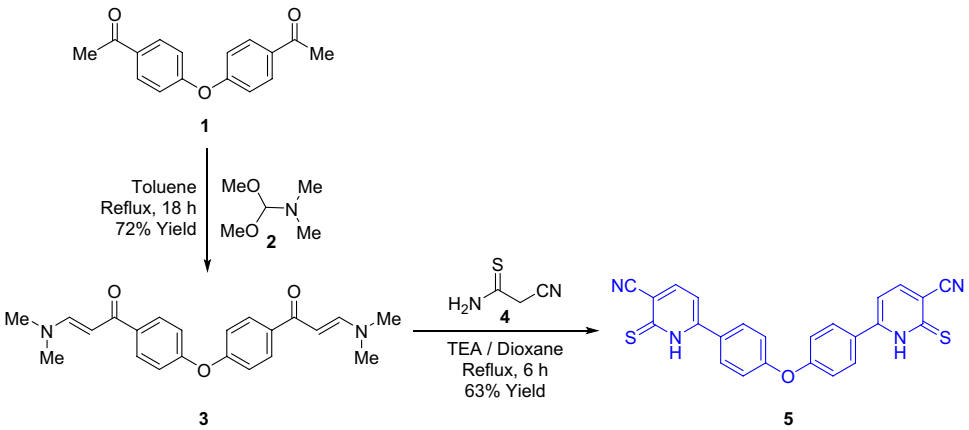


Fig. 2 Examples of biologically active pyridine-fused pyrazoles, and thiophenes



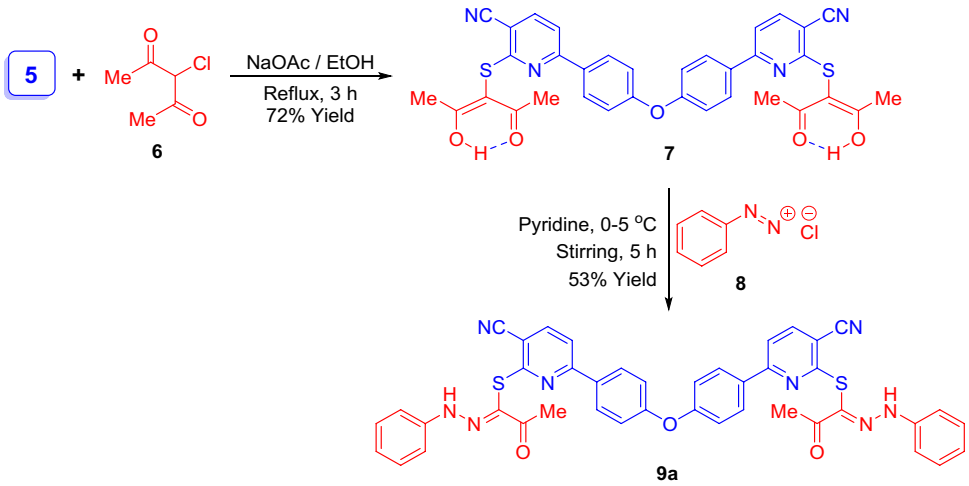
Scheme 1 Synthesis of bis(2-thioxo-1,2-dihydropyridine-3-carbonitrile) **5**

Following that, sodium acetate-mediated reaction of bis(pyridine-2(1*H*)-thione) **5** in ethanol at reflux for 3 h with two equivalents of 3-chloropentane-2,4-dione **6** was carried out. The previous alkylation yielded the corresponding bis(2-((2-oxopropyl)thio)nicotinonitrile) **7** in 72% yield [40]. As detected by ^{1}H-NMR spectrum of product **7**, the enol form predominates over the keto form in solution. The aforementioned finding is consistent with previous articles reported by Hu et al. and Chen et al. regarding the alkylation of thiophenols using 3-chloropentane-2,4-dione. [49, 50] The Japp-Klingemann reaction of the prior bis(nicotinonitrile) with benzene diazonium chloride **8** in pyridine at 0–5 °C yielded the bis(hydrazonothioate) **9a** in 53% yield (see Scheme 2) [51]. The IR spectrum of such a product showed three absorption bands at 3263, 2213 and 1669 cm^{-1} corresponding to NH, CN and CO function groups. Additionally, its ^{1}H-NMR spectrum revealed two singlet signals at δ 2.60 and 11.04 corresponding to $COCH_3$, NH protons and four doublet signals at δ 7.11, 7.75, 8.05 and 8.10 due to the protons of, oxybis(phenylene) linker, pyridine-H4 and H5 groups. Furthermore, it showed two triplet and one doublet signals at δ 7.03, 7.34 and 7.47 corresponding to the protons of two phenyl units (see Experimental section).

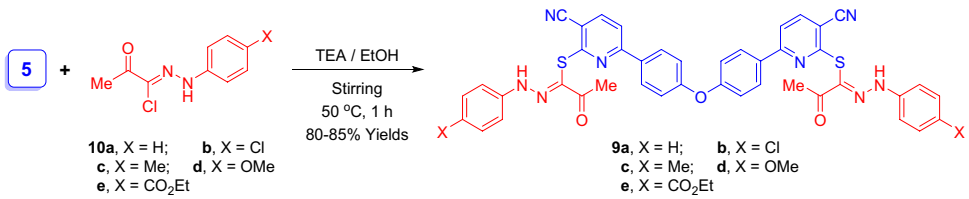
Another synthetic protocol was used to prepare bis(hydrazonothioate) **9a** in a more efficient yield. Thus, bis(pyridine-2(1*H*)-thione) **5** reacted with two equivalents of hydrazonyl chloride **10a**. The reaction was mediated by triethylamine and conducted in ethanol for 1 h with constant stirring at 50 °C. The prior protocol afforded the same **9a** in all aspects with 84% yield [52]. Using the same condition, a series of bis(hydrazonothioates) **9b-9e** was prepared utilizing the appropriate hydrazonyl chloride **10b–10e** (see Scheme 3 and Experimental section).

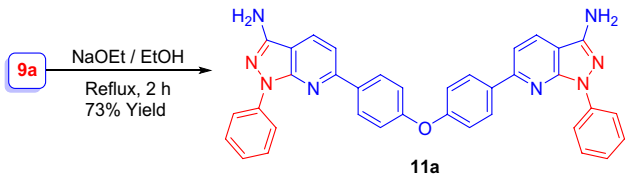
Next, heating of bis(hydrazonothioate) **9a** in ethanolic sodium ethoxide solution at reflux for 2 h afforded a sole

Scheme 2 Synthesis of bis(hydrazonothioate) **9a**



Scheme 3 Synthesis of bis(hydrazonothioates) **9b–9e**





Scheme 4 Synthesis of bis(pyrazolo[3,4-*b*]pyridine) **11a**

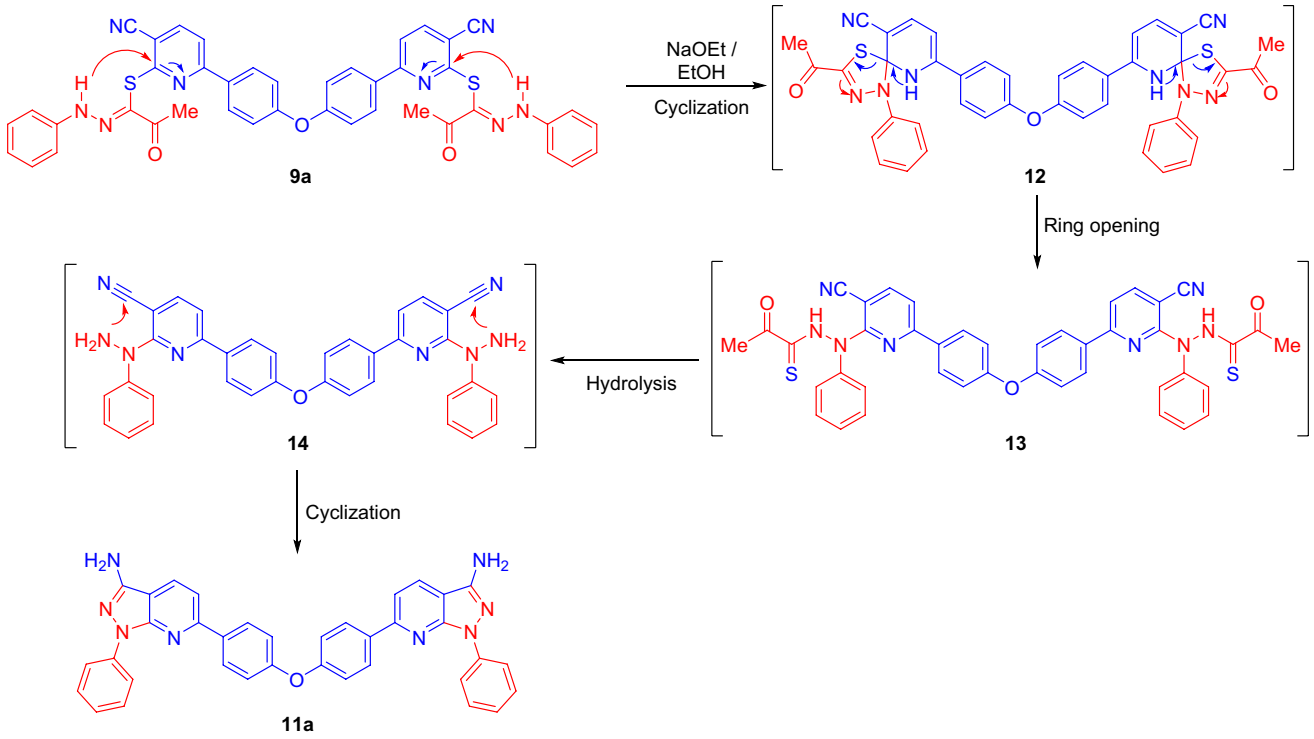
product as detected by TLC analysis. The structure of the previous product was elucidated by considering its elemental analysis and spectral data. Its mass spectrum revealed an intese molecular ion peak at $m/z = 586$. Furthermore, its IR spectrum showed the absence of nitrile or carbonyl functions, with characteristic bands due to amino groups appearing at 3453 and 3290 cm^{-1}. Additionally, its NMR spectrum revealed the absence of acetyl or NH protons, with a singlet signal at δ 5.13 owing to amino groups (see Experimental section). The data presented above established the formation of the target bis(pyrazolo[3,4-*b*]pyridine) **11a**, which was isolated in 73% yield (see Scheme 4) [52].

The conversion of bis(hydrazonothioate) **9a** into the bis(pyrazolo[3,4-*b*]pyridine) **11a** could be followed by a Smiles-type reaction [53] to yield bis(thiohydrazide) **13** through the spiro-intermediate **12**. Following that, **13** was readily hydrolyzed by sodium ethoxide and then underwent cyclization [54] to yield the target **11a** via the intermediate **14** (see Scheme 5) [52].

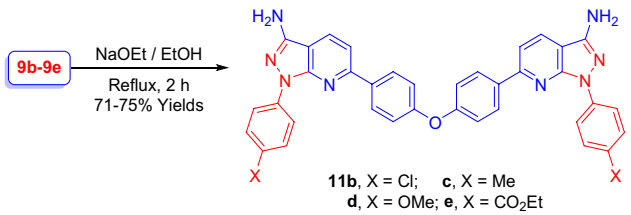
A new series of bis(pyrazolo[3,4-*b*]pyridines) **11b–11e** was prepared, in good yields, using the previous protocol and the appropriate bis(hydrazonothioates) **9b–9e** (see Scheme 6 and Experimental section).

The structure of the target bis(pyrazolo[3,4-*b*]pyridine) **11a** was more confirmed by its independent synthesis via a different route. Therefore, compound **5** reacted with two equivalents of hydrazonyl chloride **15** to yield the bis(hydrazonothioate) **16**. Heating of the previous compound in ethanolic sodium ethoxide solution afforded a product that was identical to **11a** with 65% yield (see Scheme 7 and Experimental section).

Another goal of this study was to prepare a new series of the bis(thieno[2,3-*b*]pyridines) **20**. Therefore. Compound **5** reacted with two equivalents of different α-halogenated reagents, including chloroacetone **19a**, chloroacetonitrile **19b**, chloroacetamide **19c**, ethyl chloroacetate **19d**, 2-bromo-1-phenylethanone **19e** or 2-bromo-1-(4-chlorophenyl)ethan-1-one **19f**. The reaction was carried out in ethanolic sodium ethoxide solution at reflux for 4 h to give the target **20a–20f** in 77–84% yields. It is worth noting that heating bis(nicotinonitrile) **7** in ethanolic sodium ethoxide solution at reflux for 2 h yielded a product, in 70% yield, that was identical to bis(2-acetylthieno[2,3-*b*]pyridine) **20a** in all aspects (see Scheme 8 and Experimental section) [54, 55].



Scheme 5 A plausible mechanism for the formation of bis(pyrazolo[3,4-*b*]pyridine) **11a**



Scheme 6 Synthesis of bis(pyrazolo[3,4-*b*]pyridines) **11b–11e**

Biology

The in vitro AChE inhibitory activity

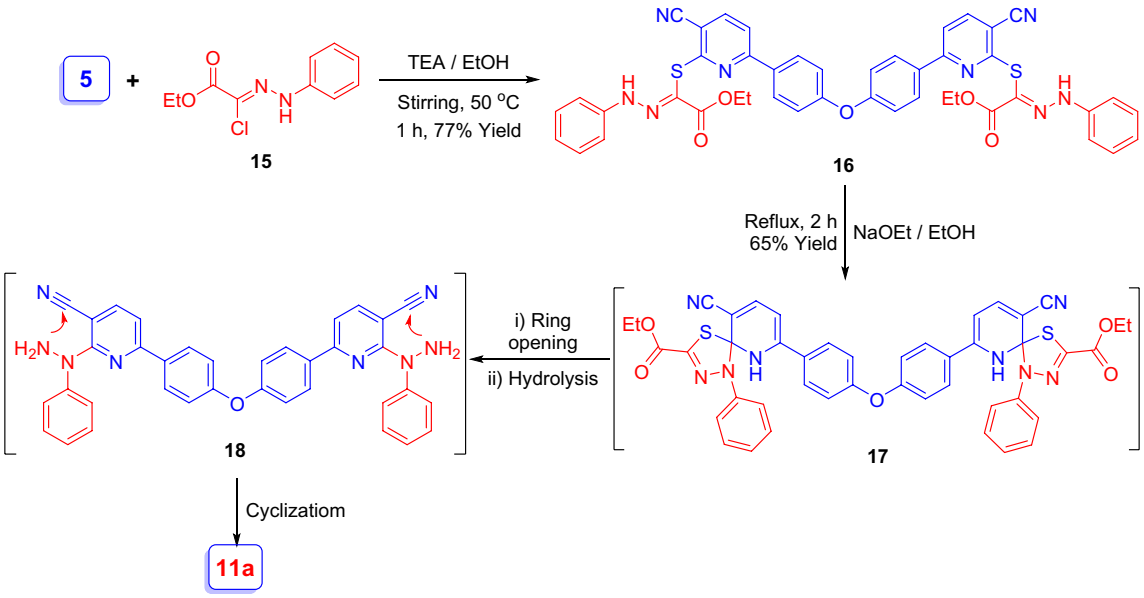
Using Ellman method and at a concentration of 100 µM, the AChE inhibitory activity was estimated as the inhibition percentage of the tested compounds. [44, 56] At the previous concentration, the reference donepezil gave inhibition percentage of 93.5 (see Table 1) [57].

In general, the tested series of bis(thieno[2,3-*b*]pyridines) **20** showed stronger inhibitory activity against AChE than the other series of bis(pyrazolo[3,4-*b*]pyridines) **11**. Therefore, **20b** and **20e**, linked to nitrile and benzoyl functions at thiophene-C2, respectively, inhibited AChE the best. The previous hybrids had percentages of inhibition of 83.2 and 78.9, respectively. Additionally, hybrids **20a**, **20c** and **20f**, linked to acetyl, carbamoyl and 4-chlorobenzoyl functions, respectively, revealed decreased AChE inhibitory activity. They had percentages of inhibition of 67.7, 55.4 and 62.1, respectively. The lowest activity is attributed to **20d**, which is linked to ethoxycarbonyl group, and has a 47.8 inhibition percentage. Only **11d**, linked to 4-methoxyphenyl unit, out of five tested bis(pyrazolo[3,4-*b*]pyridines) showed inhibition percentage greater than 50%. The previous hybrid had a inhibition percentage of 52.3, while other tested hybrids in bis(pyrazolo[3,4-*b*]pyridines) **11a**, **11b**, **11c** and **11e** had percentages of inhibition ranging from 15.2 to 37.4.

The structure–activity relationship study

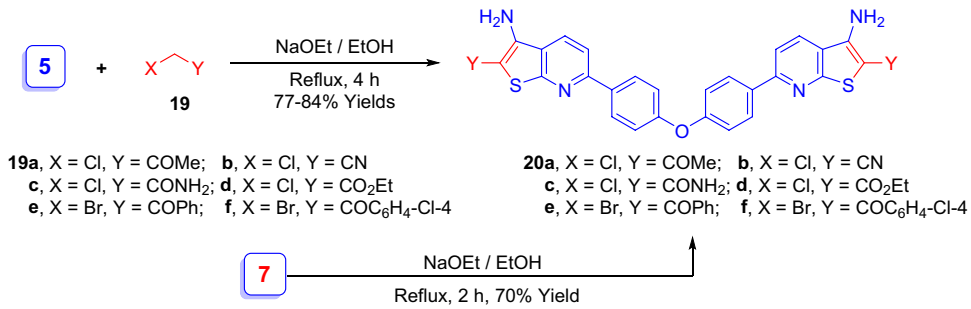
The goal of this study was to prepare two series of bis(pyrazolo[3,4-*b*]pyridine) **11** and bis(thieno[2,3-*b*]pyridines) **20** as potential AChE inhibitors. The first prepared series was attached to an aryl group at N1 and linked to a substituent with different electronic properties at para-position. The other prepared series was attached to different functions at thiophene-C2. To shed more light about the significance of the aforementioned results, Table 2 lists the inhibitory activity of some related and promising pyridine-fused pyrazoles as well as thiophenes.

Regarding the pyrazolo[3,4-*b*]pyridine hybrids, hybrid **I** with unsubstituted pyrazole-NH demonstrated the best AChE inhibitory activity with a nano-scale IC_{50} of 4.8 nM (see Table 2, Entry 1) [18]. Subtitution of pyrazole-NH



Scheme 7 Alternative route for the synthesis of bis(pyrazolo[3,4-*b*]pyridine) **11a**

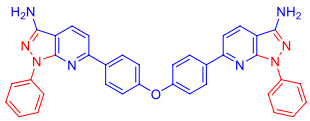
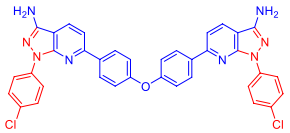
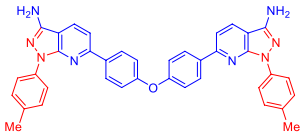
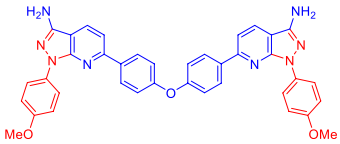
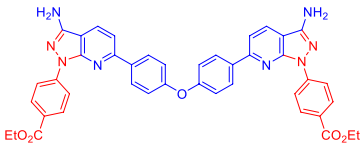
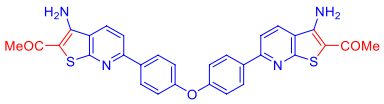
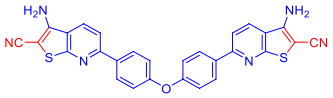
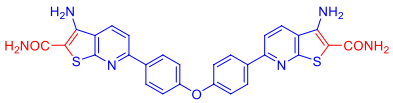
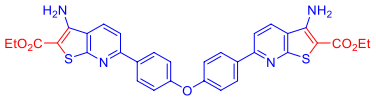
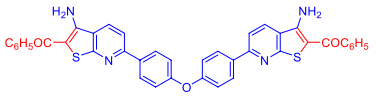
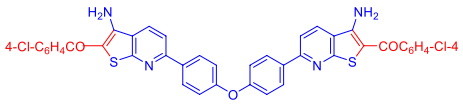
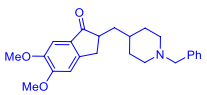
Scheme 8 Synthesis of bis(thieno[2,3-*b*]pyridines) **20a–20f**



with akyl units resulted in the decreased activity of hybrids **II** and **III** with micro-scale IC_{50} in the range of 0.0125 to 1.70 µM (see Table 2, Entries 2 and 3) [58, 59]. Furthermore, hybrid **IV** with a phenyl-linked pyrazole unit demonstrated the least potency with IC_{50} of 28.5 µM (see Table 2, Entry 4) [60]. However, the presence of electron releasing units such as methyl or methoxy groups attached to the arene units resulted in improving the inhibitory activity of the respective hybrids. Thus, arene-linked bis(pyrazole) hybrids **V**, attached to 4-OMe and Me units, demonstrated moderate activity with inhibtion precentages of 54.7 and 50.8 when tested at 100 µM (see Table 2, Entry 5) [42]. These findings are consistent with the findings of the current study, in which bis(pyrazole) hybrids **11** showed a diverse potency with inhibition percentages ranging from 15.2 to 52.3. The highest activity in series **11** was obtained with those attached to 4-methoxyphenyl (**11d**, inhibition percentage of 52.3) or *p*-tolyl units (**11c**, inhibition precentage of 37.4) (see Table 2, Entry 6). Additionally, hybrid **11e** attached to the strong electron withdrawing 4-ethoxycarbonyl group demonstrated the least activity with inhibition percentage of 15.2.

In terms of the thieno[2,3-*b*]pyridines, hybrid **VI** attached to alkyl residue at thiophene-C2 and C3 displayed excellent potency with IC_{50} of 1.55 µM (see Table 2, Entry 7). [20] Several publications reported the suprior inhibitory activity of hybrids **VII**, especially those attached to nitrile or benzoyl functions at thiophene-C2 (see Table 2, Entry 8). [61, 62] Ahmed et al. [42] reported similar results in its work on piperazine-linked thieno[2,3-*b*]pyridines **VIII**, where those hybrids attached to nitrile and benzoyl functions demonstrated the best potency with inhibitory percentages of 88.4 and 79.9, respectively, at 100 µM (see Table 2, Entry 9). The previous results support the interesting activity of nitrile or benzoyl-linked hybrids **20b(20e)** in the current study. The hybrids **20b(20e)** displayed inhibition percentages of 83.2 and 78.9, respectively (see Table 2, Entry 10). All results are summarized in Fig. 3.

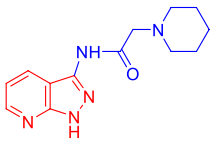
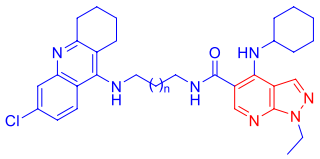
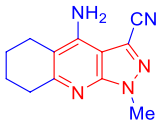
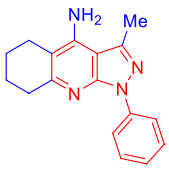
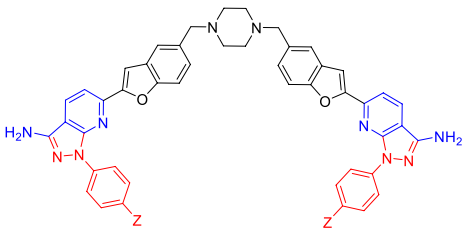
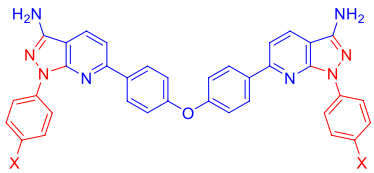
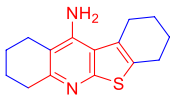
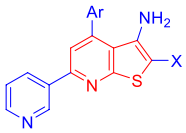
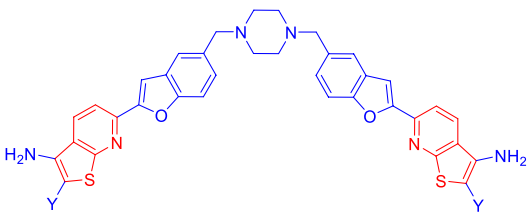
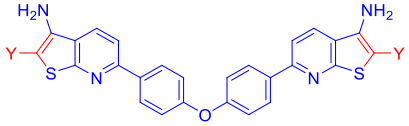
Table 1 The AChE percentages of inhibition of the new bis(pyrazolo[3,4-*b*]pyridines) **11** and bis(thieno[2,3-*b*]pyridines) **20** at a concentration of 100 µM (negative control was taken as 100% inhibition)

Entry	Product	Structure	Name	Inhibition%
1	11a		6,6'-(Oxybis(4,1-phenylene))bis(1-phenyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-amine)	23.5
2	11b		6,6'-(Oxybis(4,1-phenylene))bis(1-(4-chlorophenyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-amine)	29.0
3	11c		6,6'-(Oxybis(4,1-phenylene))bis(1-(*p*-tolyl)-1*H*-pyrazolo[3,4-*b*]pyridin-3-amine)	37.4
4	11d		6,6'-(Oxybis(4,1-phenylene))bis(1-(4-methoxyphenyl)-1*H*-pyrazolo[3,4-b]pyridin-3-amine)	52.3
5	11e		Diethyl 4,4'-((oxybis(4,1-phenylene))bis(3-amino-1*H*-pyrazolo[3,4-*b*]pyridine-6,1-diyl))dibenzoate	15.2
6	20a		6,6'-(Oxybis(4,1-phenylene))bis(2-acetyl-3-aminothieno[2,3-*b*]pyridine)	67.7
7	20b		6,6'-(Oxybis(4,1-phenylene))bis(3-aminothieno[2,3-*b*]pyridine-2-carbonitrile)	83.2
8	20c		6,6'-(Oxybis(4,1-phenylene))bis(3-aminothieno[2,3-*b*]pyridine-2-carboxamide)	55.4
9	20d		Diethyl 6,6'-(oxybis(4,1-phenylene))bis(3-aminothieno[2,3-*b*]pyridine-2-carboxylate)	47.8
10	20e		6,6'-(Oxybis(4,1-phenylene))bis(3-amino-2-benzoylthieno[2,3-*b*]pyridine)	78.9
11	20f		6,6'-(Oxybis(4,1-phenylene))bis(3-amino-2-(4-chlorobenzoyl)thieno[2,3-*b*]pyridine)	62.1
12	Reference		Donepezil	93.5

DPPH antioxidant activity

Numerous research works have been carried out to examine the effectiveness of antioxidant therapeutic strategies in the treatment of Alzheimer's disease [63]. A subset of amyloid plaques has recently been shown to yield free radicals in living, Alzheimer's models, and human Alzheimer's tissues. Therapy with antioxidants can be used to

Table 2 Comparison between the AChE inhibitory activity of some related and promising pyridine-fused pyrazoles as well as thiophenes **I–VIII** and the new products **11(20)**

Entry	Hybrid	Structure	Activity	Reference
1	I		IC_{50} = 4.8 nM	Ref. [58]
2	II		IC_{50} = 0.125 µM	Ref. [59]
3	III		IC_{50} = 1.70 µM	Ref. [60]
4	IV		IC_{50} = 28.5 µM	Ref. [61]
5	V		Best activity *Z* = Me(OMe) (inhibition% of 50.8(54.7) at 100 µM)	Ref. [42]
6	11c(11d)		Best activity **11c(11d)**, *X* = Me(OMe) (inhibition% of 37.4(52.3) at 100 µM)	Current work
7	VI		IC_{50} = 1.55 µM	Ref. [62]
8	VII		Best activity *X* = CN(COPh)	Ref. [63, 64]
9	VIII		Best activity *Y* = CN(COPh) (inhibition% of 88.4(79.9) at 100 µM)	Ref. [42]
10	20b(20e)		Best activity **20b(20e)**, *Y* = CN(COPh) (inhibition% of 83.2(78.9) at 100 µM)	Current work

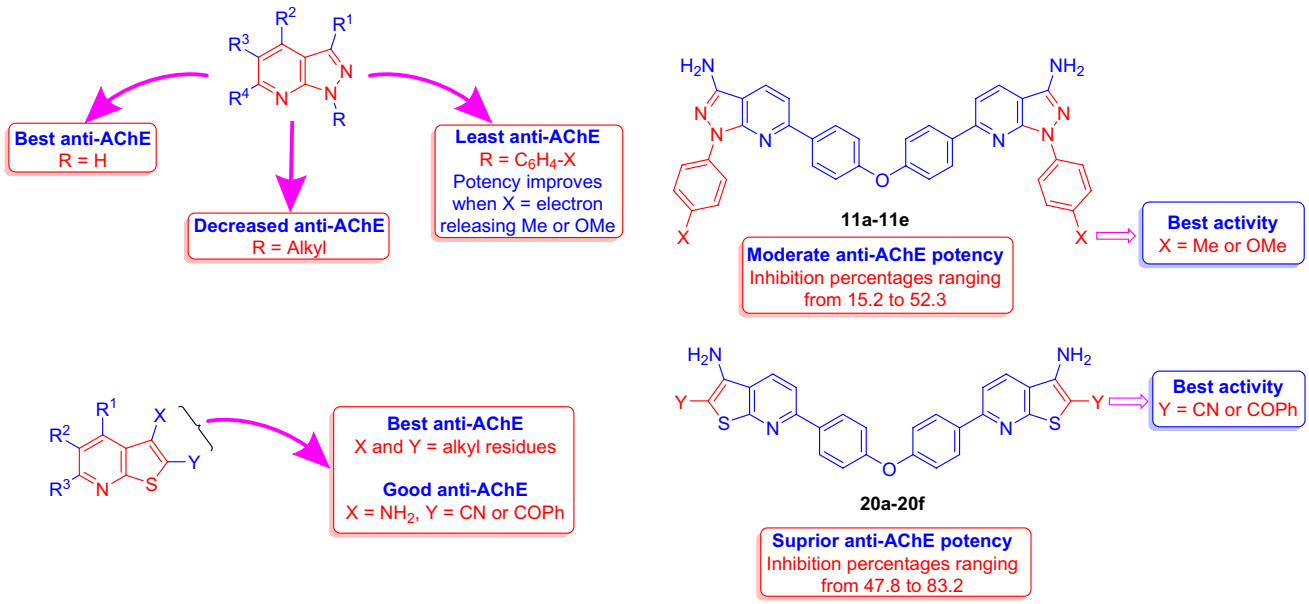


Fig. 3 Structure–activity relationship of the new pyridine-fused hybrids **11(20)**

neutralize such highly reactive molecules, providing therapeutic efficacy in Alzheimer's disease [64]. Based on the foregoing, the antioxidant potency of the tested hybrids is thought to be beneficial for AD treatment.

The DPPH free radical quenching capability of the new hybrids **11(20)** was examined [45]. The results are recorded as the inhibition percentage of the new hybrids at a concentration of 25 µg/mL using ascorbic acid as a reference with inhibition percentage of 88.7 (see Table 3). The bis(thieno[2,3-*b*]pyridine) **20b** had the highest antioxidant activity, with an inhibition percentage of 82.6. Furthermore, the bis(thieno[2,3-*b*]pyridines) **20e**, **20f** and **20a** exhibited decreased free radical quenching activities with percentages of inhibition of 69.8, 60.2 and 54.2. Furthermore, bis(thieno[2,3-*b*]pyridines **20c** and **20d**, as well as all bis(pyrazolo[3,4-*b*]pyridines) **11a–11e** tested, demonstrated lower efficacies, with inhibition percentages less than 50%. The previous hybrids had inhibition percentages ranging from 7.7 to 39.3. The superior antioxidant activity of nitrile-linked hybrid **20b** is consistent with previously reported findings on thieno[2,3-*b*]pyridine analogues by Ahmed et al. [42] and Çakmak el al [65].

SwissADME predictions of new hybrids

The physicochemical properties, lipophilicity, and drug likeness of the new products were predicted using SwissADME (see Table S1 in the electronic supplementary file). The molecular weights (MW) of bis(pyrazolo[3,4-*b*]pyridines) **11** ranged from 586.64 to 730.77 g/mol, with a number of rotatable bonds (RB), and H-bond acceptors (HA) in the ranges of 6–12, and 5–9, respectively, and H-bond donors (HD) of 2. Moreover, the previous hybrids showed topological polar surface area (TPSA) in the range of 117.19 to 169.79 Å^2. The partition coefficient between octanol and water (log $P_{o/w}$) was also predicted using SwissADME as a lipophilicity descriptor, which is important for drug development [66]. The log $P_{o/w}$ values of bis(pyrazolo[3,4-*b*]pyridines) **11** ranged from 4.81 to 6.34. Furthermore, the MW of bis(thieno[2,3-*b*] pyridines) **20** ranged from 516.60 to 743.68 g/mol, with a number of RB, HA and HD in the ranges of 4–10, 5–7 and 2–4, respectively. In addition, the log $P_{o/w}$ and TPSA values of the previous hybrids ranged from 1.51 to 4.68 and 120.13 to 172.17 Å^2.

Table 3 DPPH scavenging activity of the new bis(pyrazolo[3,4-*b*]pyridines) **11** and bis(thieno[2,3-*b*]pyridines) **20** at a concentration of 25 µg/mL (negative control was taken as 100% inhibition)

	11a	11b	11c	11d	11e	20a	20b	20c	20d	20e	20f	Ascorbic acid
Inhibition percentage	7.7	7.9	11.3	20.5	6.4	54.2	82.6	39.3	37.5	69.8	60.2	88.7

According to Lipinski's rule of five, orally active drugs must not violate more than one of the following physicochemical properties: MW ≤ 500, their RB ≤ 10, HD ≤ 5, HA ≤ 10 and log $P_{o/w} \leq 5$ [67]. Based on the previous rule, hybrid bis(pyrazolo[3,4-*b*]pyridine) **11d**, like bis(thieno[2,3-*b*]pyridines) **20a–20f**, violate only one parameter (MW > 500) and are thus considered drug-like. Other hybrids are not classified as drug-like scaffolds because hybrids **11a–11c** violate two parameters (MW > 500 and log $P_{o/w} > 5$), while hybrid **11e** violates three parameters (MW > 500, RB > 10, and log $P_{o/w} > 5$).

Conclusion

The synthon bis(pyridine-2(1*H*)-thione) was used to prepare new seires of bis(pyrazolo[3,4-*b*]pyridines) **11** and bis(thieno[2,3-*b*]pyridines) **20**, in good to excellent yields. Generally, the tested series of bis(thieno[2,3-*b*] pyridines) **20** showed stronger AChE inhibitory activity as well as DPPH antioxidant activity than the other series of bis(pyrazolo[3,4-*b*]pyridines) **11**. The bis(thieno[2,3-*b*] pyridine-2-carbonitrile) **20b** showed the best AChE inhibitory and DPPH antioxidant activity with inhibition percentages of 83.2 and 82.6, respectively. SwissADME was used to predict that hybrid bis(pyrazolo[3,4-*b*]pyridine) **11d,** as well as bis(thieno[2,3-*b*]pyridines) **20,** is considered drug-like.

Experimental

Materials

All solvents were acquired from commercial sources and used as received unless otherwise stated. All other chemicals were acquired from Merck or Aldrich. These chemicals were used without further purification. The melting points were measured on a Stuart melting point apparatus and are uncorrected. IR spectra were recorded on a Smart iTR, which is an ultra-high-performance, versatile attenuated total reflectance (ATR) sampling accessory on the Nicolet iS10 FT-IR spectrometer manufactured. NMR spectra were recorded on a Bruker Avance III 400 spectrometer (400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR) using TMS as an internal standard and DMSO-d_6 as solvent and chemical shifts were expressed as δ ppm units. Mass spectra were recorded on a GC–MS-QP1000EX spectrometer using inlet type at 70 eV. Elemental analyses were carried out on a EuroVector instrument C, H, N analyzer EA3000 Series.

The procedures and spectral data

Synthesis of 6,6'-(oxybis(4,1-phenylene)) bis(2-thioxo-1,2-dihydropyridine-3-carbonitrile) (5)

A mixture of bis(enaminone) 3 (5 mmol), and 2-cyanothioacetamide 4 (10 mmol) in the presence of triethylamine (1 mL) in dioxane (20 mL) was heated at reflux for 6 h. The obtained product was filtered, washed with ethanol and recrystallized from dioxane/ethanol mixture as orange solid (63%); m.p. 262–263 °C; IR (υ cm^{-1}): 3382 (NH), 2219 (CN); ^{1}H-NMR (DMSO-d_6): δ 7.08–7.11 (m, 6H, 4 ArH and 2 pyridine-H5), 7.79 (d, *J* = 8.8 Hz, 4H, ArH), 8.09 (d, *J* = 5.6 Hz, 2H, 2 pyridine-H4), 14.08 (br s, 2H, 2 NH); ^{13}C-NMR (DMSO-d_6): δ 110.6, 114.9, 117.1, 120.9, 128.7, 131.5, 140.6, 147.6, 159.3, 179.7; Anal. for $C_{24}H_{14}N_4OS_2$ (438.5) Calcd: C, 65.74; H, 3.22; N, 12.78; found: C, 65.86; H, 3.13; N, 12.69%.

Synthesis of 6,6'-(oxybis(4,1-phenylene))bis(2-((2,4-dioxopentan-3-yl)thio)nicotinonitrile) (7)

A mixture of bis(pyridine-2(1*H*)-thione) 5 (5 mmol), 3-chloropentane-2,4-dione 6 (10 mmol) and anhydrous sodium acetate (12 mmol) in ethanol (15 mL) was heated at reflux for 3 h. The product was collected by filtration, washed with water, ethanol, dried and recrystallized from dioxane/ethanol mixture as colorless solid (dioxane/ethanol mixture; 72%); m.p. 176–178 °C; IR (υ cm^{-1}): 2210 (CN), 1723 (CO); ^{1}H-NMR (DMSO-d_6): δ 2.32 (s, 6H, 2 CH_3), 2.38 (s, 6H, 2 CH_3), 7.11 (d, *J* = 8.8 Hz, 4H, ArH), 7.77 (d, *J* = 5.2 Hz, 2H, 2 pyridine-H5), 8.07–8.11 (m, 6H, 4 ArH and 2 pyridine-H4), 16.78 (s, 2H, 2 OH); ^{13}C-NMR (DMSO-d_6): δ 18.4, 26.5, 99.8, 106.1, 114.8, 116.7, 120.4, 128.9, 131.8, 141.5, 159.2, 159.6, 162.6, 175.2, 195.6; Anal. for $C_{34}H_{26}N_4O_5S_2$ (634.7) Calcd: C, 64.34; H, 4.13; N, 8.83; found: C, 64.19; H, 3.99; N, 9.04%.

General procedure for the synthesis of bis(hydrazonothioates) 9 and 16

Method 'A' Over a period of 1 h at 0–5 °C, benzene diazonium chloride **8** (10 mmol) was added to a solution of bis(nicotinonitrile) **7** (5 mmol) in pyridine (20 mL). In an ice bath, the mixture was stirred for an additional 5 h. The mixture was then placed in the refrigerator for 5 h. The solid obtained was filtered, washed with water, dried, and recrystallized from dioxane/ethanol mixture to yield **9a**.

Method 'B' A mixture of bis(pyridine-2(1*H*)-thione) **5** (5 mmol), each of the appropriate hydrazonyl chlorides **10a–10e** or **15** (10 mmol) and triethylamine (10 mmol) in ethanol (20 mL) was stirred at 50 °C for 1 h. The reaction mixture was cooled and the obtained product was filtrated,

washed with ethanol, and recrystallized from the appropriate solvent.

(Oxybis(4,1-phenylene))bis(3-cyanopyridine-6,2-diyl) bis(2-oxo-N-phenylpropanehydrazonothioate) (9a)

Colorless solid (dioxane/ethanol mixture; 53% using Method 'A'; 84% using Method 'B'); m.p. 226–229 °C; IR (υ cm^{-1}): 3263 (NH), 2213 (CN), 1669 (CO); ^{1}H-NMR (DMSO-d_6): δ 2.60 (s, 6H, 2 $COCH_3$), 7.03 (t, J = 7.6 Hz, 2H, ArH), 7.11 (d, J = 8.8 Hz, 4H, ArH), 7.34 (t, J = 7.6 Hz, 4H, ArH), 7.47 (d, J = 7.6 Hz, 4H, ArH), 7.75 (d, J = 5.2 Hz, 2H, 2 pyridine-H5), 8.05 (d, J = 8.8 Hz, 4H, ArH), 8.10 (d, J = 5.2 Hz, 2H, 2 pyridine-H4), 11.04 (s, 2H, 2 NH); ^{13}C-NMR (DMSO-d_6): δ 25.4 (CH_3), 104.4 (pyridine-*C*3), 114.9 (Linker-C2,6), 115.1 (*C*N), 115.2 (Ar–*C*), 120.0 (pyridine-*C*5), 120.2 (Ar–*C*), 128.7 (Linker-C3,5), 129.1 (Ar–*C*), 131.6 (Linker-C4), 138.8 (H*C* = N), 141.9 (pyridine-*C*4), 142.5 (Ar–*C*), 157.2 (pyridine-*C*6), 159.6 (Linker-C1), 162.4 (pyridine-*C*2), 191.6 (*C*O); MS *m/z* (%): 758 (M^+, 42.4); Anal. for $C_{42}H_{30}N_8O_3S_2$ Calcd: C, 66.47; H, 3.98; N, 14.77; found: C, 66.56; H, 4.11; N, 14.95%.

(Oxybis(4,1-phenylene))bis(3-cyanopyridine-6,2-diyl) bis(N-(4-chlorophenyl)-2-oxopropanehydrazonothioate) (9b)

Colorless solid (dioxane/ethanol mixture; 83%); m.p. 232 °C; IR (υ cm^{-1}): 3262 (NH), 2212 (CN), 1670 (CO); ^{1}H-NMR (DMSO-d_6): δ 2.59 (s, 6H, 2 $COCH_3$), 7.09 (d, J = 8.8 Hz, 4H, ArH), 7.42 (d, J = 7.6 Hz, 4H, ArH), 7.62 (d, J = 7.6 Hz, 4H, ArH), 7.72 (d, J = 5.2 Hz, 2H, 2 pyridine-H5), 8.04 (d, J = 8.8 Hz, 4H, ArH), 8.12 (d, J = 5.2 Hz, 2H, 2 pyridine-H4), 11.10 (s, 2H, 2 NH); ^{13}C-NMR (DMSO-d_6): δ 25.6, 104.2, 114.8, 115.5, 116.7, 120.4, 128.7, 129.1, 131.5, 132.2, 139.0, 140.8, 141.7, 156.5, 159.6, 161.9, 191.8; Anal. for $C_{42}H_{28}Cl_2N_8O_3S_2$ (827.7) Calcd: C, 60.94; H, 3.41; N, 13.54; found: C, 61.12; H, 3.24; N, 13.35%.

(Oxybis(4,1-phenylene))bis(3-cyanopyridine-6,2-diyl) bis(2-oxo-N-(p-tolyl)propanehydrazonothioate) (9c)

Colorless solid (dioxane; 80%); m.p. 236 °C; IR (υ cm^{-1}): 3247 (NH), 2212 (CN), 1674 (CO); ^{1}H-NMR (DMSO-d_6): δ 2.26 (s, 6H, 2 *p*-CH_3), 2.59 (s, 6H, 2 $COCH_3$), 7.11–7.14 (m, 8H, ArH), 7.43 (d, J = 7.6 Hz, 4H, ArH), 7.71 (d, J = 5.2 Hz, 2H, 2 pyridine-H5), 8.08–8.11 (m, 6H, 4 ArH and 2 pyridine-H4), 10.98 (s, 2H, 2 NH); ^{13}C-NMR (DMSO-d_6): δ 20.2, 25.4, 104.4, 114.5, 115.2, 115.4, 120.1, 128.6, 129.5, 131.9, 132.1, 138.8, 140.2, 141.4, 156.7, 158.8, 160.9, 191.6; MS *m/z* (%): 786 (M^+, 27.5); Anal. for $C_{44}H_{34}N_8O_3S_2$ Calcd: C, 67.16; H, 4.36; N, 14.24; found: C, 66.89; H, 4.60; N, 14.47%.

(Oxybis(4,1-phenylene))bis(3-cyanopyridine-6,2-diyl) bis(N-(4-methoxyphenyl)-2-oxopropanehydrazonothioate) (9d)

Colorless solid (dioxane/ethanol mixture; 85%); m.p. 220–221 °C; IR (υ cm^{-1}): 3253 (NH), 2210 (CN), 1665 (CO); ^{1}H-NMR (DMSO-d_6): δ 2.58 (s, 6H, 2 $COCH_3$), 3.78 (s, 6H, 2 OCH_3), 6.93 (d, J = 7.6 Hz, 4H, ArH), 7.09 (d, J = 8.8 Hz, 4H, ArH), 7.50 (d, J = 7.6 Hz, 4H, ArH), 7.77 (d, J = 5.6 Hz, 2H, 2 pyridine-H5), 8.09–8.12 (m, 6H, 4 ArH and 2 pyridine-H4), 10.98 (s, 2H, 2 NH); ^{13}C-NMR (DMSO-d_6): δ 25.5, 55.4, 104.4, 113.8, 114.8, 115.9, 120.0, 120.8, 128.6, 132.4, 136.3, 139.0, 140.1, 155.7, 157.9, 159.4, 161.3, 191.6; Anal. for $C_{44}H_{34}N_8O_5S_2$ (818.9) Calcd: C, 64.53; H, 4.18; N, 13.68; found: C, 64.31; H, 4.02; N, 13.91%.

(Oxybis(4,1-phenylene))bis(3-cyanopyridine-6,2-diyl) bis-(N-(4-ethoxycarbonylphenyl)-2-oxopropanehydrazonothioate) (9e)

Colorless solid (dioxane; 81%); m.p. 228 °C; IR (υ cm^{-1}): 3256 (NH), 2215 (CN), 1707, 1652 (2 CO); ^{1}H-NMR (DMSO-d_6): δ 1.32 (t, 6H, J = 7.2 Hz, 2 OCH_2CH_3), 2.63 (s, 6H, 2 $COCH_3$), 4.29 (q, 4H, J = 7.2 Hz, 2 OCH_2CH_3), 7.07 (d, J = 8.8 Hz, 4H, ArH), 7.62 (d, J = 7.6 Hz, 4H, ArH), 7.73 (d, J = 5.6 Hz, 2H, 2 pyridine-H5), 7.83 (d, J = 7.6 Hz, 4H, ArH), 8.04 (d, J = 8.8 Hz, 4H, ArH), 8.10 (d, J = 5.6 Hz, 2H, 2 pyridine-H4), 11.27 (s, 2H, 2 NH); ^{13}C-NMR (DMSO-d_6): δ 13.9, 25.4, 61.0, 104.2, 114.9, 115.5, 115.8, 120.2, 123.4, 128.6, 131.5, 131.9, 138.9, 141.3, 147.7, 156.6, 158.6, 160.8, 166.2, 191.7; Anal. for $C_{48}H_{38}N_8O_7S_2$ (903.0) Calcd: C, 63.85; H, 4.24; N, 12.41; found: C, 63.93; H, 4.10; N, 12.57%.

Diethyl 2,2'-(((oxybis(4,1-phenylene))bis(3-cyanopyridine-6,2-diyl))bis(sulfanediyl))bis(2-(2-phenylhydrazineylidene)acetate) (16)

Colorless solid (dioxane/ethanol mixture; 77%); m.p. 214–215 °C; IR (υ cm^{-1}): 3267 (NH), 2212 (CN), 1699 (CO); ^{1}H-NMR (DMSO-d_6): δ 1.16 (t, 6H, J = 7.2 Hz, 2 OCH_2CH_3), 4.20 (q, 4H, J = 7.2 Hz, 2 OCH_2CH_3), 7.02–7.09 (m, 6H, ArH), 7.36 (t, J = 7.6 Hz, 4H, ArH), 7.48 (d, J = 7.6 Hz, 4H, ArH), 7.70 (d, J = 5.2 Hz, 2H, 2 pyridine-H5), 8.07–8.10 (m, 6H, 4 ArH and 2 pyridine-H4), 10.90 (s, 2H, 2 NH); ^{13}C-NMR (DMSO-d_6): δ 13.9, 61.0, 104.2, 114.8, 115.3, 115.6, 120.4, 120.5, 128.5, 129.2, 132.1, 138.8, 140.4, 142.4, 156.8, 158.7, 161.7, 162.4; Anal. for $C_{44}H_{34}N_8O_5S_2$ (818.9) Calcd: C, 64.53; H, 4.18; N, 13.68; found: C, 64.62; H, 4.07; N, 13.54%.

General procedure for the synthesis of bis(pyrazolo[3,4-b] pyridines) 11

A mixture of bis(hydrazonothioates) **9** or **16** (5 mmol) in ethanolic sodium ethoxide solution (prepared by dissolving 12 mmol of sodium in 15 mL of ethanol) was heated at reflux for 2 h. The product was collected by filtration, washed with ethanol, dried and recrystallized from the appropriate solvent.

6,6'-(Oxybis(4,1-phenylene))bis(1-phenyl-1H-pyrazolo[3,4-b]pyridin-3-amine) (11a)

Yellow solid (dioxane/ethanol mixture; 73% using Method 'A'; or 65% using Method 'B'); m.p. 248–249 °C; IR (υ cm^{-1}): 3453, 3290 (NH_2); ^{1}H-NMR (DMSO-d_6): δ 5.13 (s, 4H, 2 NH_2), 7.09 (d, J = 8.8 Hz, 4H, ArH), 7.21 (t, J = 7.6 Hz, 2H, ArH), 7.40 (t, J = 7.6 Hz, 4H, ArH), 7.55 (d, J = 7.6 Hz, 4H, ArH), 7.68 (d, J = 5.2 Hz, 2H, 2 pyridine-H5), 8.12 (d, J = 8.8 Hz, 4H, ArH), 8.40 (d, J = 5.2 Hz, 2H, 2 pyridine-H4); ^{13}C-NMR (DMSO-d_6): δ 107.2, 115.3, 115.6, 119.3, 120.2, 123.5, 128.6, 128.8, 132.5, 138.4, 139.7, 150.7, 155.6, 158.5; MS *m/z* (%): 586 (M^+, 40.3); Anal. for $C_{36}H_{26}N_8O$ Calcd: C, 73.70; H, 4.47; N, 19.10; found: C, 73.43; H, 4.68; N, 18.84%.

6,6'-(Oxybis(4,1-phenylene))bis(1-(4-chlorophenyl)-1H-pyrazolo[3,4-b]pyridin-3-amine) (11b)

Yellow solid (dioxane/ethanol mixture; 71%); m.p. 244 °C; IR (υ cm^{-1}): 3440, 3294 (NH_2); ^{1}H-NMR (DMSO-d_6): δ 5.20 (s, 4H, 2 NH_2), 7.10 (d, J = 8.8 Hz, 4H, ArH), 7.53 (d, J = 7.6 Hz, 4H, ArH), 7.65 (d, J = 5.6 Hz, 2H, 2 pyridine-H5), 8.08–8.11 (m, 8H, ArH), 8.35 (d, J = 5.6 Hz, 2H, 2 pyridine-H4); ^{13}C-NMR (DMSO-d_6): δ 107.0, 115.2, 115.4, 119.6, 121.2, 128.8, 129.2, 132.3, 132.5, 138.2, 138.7, 149.3, 154.8, 158.2; Anal. for $C_{36}H_{24}Cl_2N_8O$ (655.5) Calcd: C, 65.96; H, 3.69; N, 17.09; found: C, 66.14; H, 3.93; N, 16.84%.

6,6'-(Oxybis(4,1-phenylene))bis(1-(p-tolyl)-1H-pyrazolo[3,4-b]pyridin-3-amine) (11c)

Yellow solid (dioxane; 73%); m.p. 240–243 °C; IR (υ cm^{-1}): 3432, 3295 (NH_2); ^{1}H-NMR (DMSO-d_6): δ 2.35 (s, 6H, 2 CH_3), 5.08 (s, 4H, 2 NH_2), 7.08 (d, J = 8.8 Hz, 4H, ArH), 7.49 (d, J = 7.6 Hz, 4H, ArH), 7.69 (d, J = 5.2 Hz, 2H, 2 pyridine-H5), 8.07 (d, J = 7.6 Hz, 4H, ArH), 8.13 (d, J = 8.8 Hz, 4H, ArH), 8.41 (d, J = 5.2 Hz, 2H, 2 pyridine-H4); ^{13}C-NMR (DMSO-d_6): δ 20.3, 106.8, 115.0, 115.5, 119.7, 128.4, 128.6, 129.2, 132.2, 132.6, 137.3, 137.7, 148.2, 153.9, 159.2; MS *m/z* (%): 614 (M^+, 33.8); Anal. for $C_{38}H_{30}N_8O$ Calcd: C, 74.25; H, 4.92; N, 18.23; found: C, 74.03; H, 5.05; N, 18.47%.

6,6'-(Oxybis(4,1-phenylene))bis(1-(4-methoxyphenyl)-1H-pyrazolo[3,4-b]pyridin-3-amine) (11d)

Yellow solid (dioxane, 72%); m.p. 258 °C; IR (υ cm^{-1}): 3448, 3299 (NH_2); ^{1}H-NMR (DMSO-d_6): δ 3.82 (s, 6H, 2 *p*-OCH_3), 5.04 (s, 4H, 2 NH_2), 7.09–7.12 (m, 8H, ArH), 7.68 (d, J = 5.6 Hz, 2H, 2 pyridine-H5), 8.06 (d, J = 7.6 Hz, 4H, ArH), 8.12 (d, J = 8.8 Hz, 4H, Ar–H's), 8.39 (d, J = 5.6 Hz, 2H, 2 pyridine-H4); ^{13}C-NMR (DMSO-d_6): δ 55.5, 106.5, 114.6, 114.8, 116.0, 118.7, 123.5, 128.7, 132.8, 134.0, 137.8, 147.9, 153.5, 157.8, 159.9; MS *m/z* (%): 646 (M^+, 22.9); Anal. for $C_{38}H_{30}N_8O_3$ Calcd: C, 70.58; H, 4.68; N, 17.33; found: C, 70.45; H, 4.84; N, 17.04%.

Diethyl 4,4'-((oxybis(4,1-phenylene)) bis(3-amino-1H-pyrazolo[3,4-b]pyridine-6,1-diyl))dibenzoate (11e)

Yellow solid (dioxane/ethanol mixture; 75%); m.p. 222–225 °C; IR (υ cm^{-1}): 3446, 3297 (NH_2), 1707 (CO); ^{1}H-NMR (DMSO-d_6): δ 1.30 (t, 6H, J = 7.2 Hz, 2 CH_2CH_3), 4.27 (q, 4H, J = 7.2 Hz, 2 CH_2CH_3), 5.12 (s, 4H, 2 NH_2), 7.10 (d, J = 8.8 Hz, 4H, ArH), 7.58 (d, J = 7.6 Hz, 4H, ArH), 7.70 (d, J = 5.6 Hz, 2H, 2 pyridine-H5), 8.06–8.09 (m, 8H, Ar–H's), 8.42 (d, J = 5.6 Hz, 2H, 2 pyridine-H4); ^{13}C-NMR (DMSO-d_6): δ 13.9, 61.0, 106.5, 114.8, 115.9, 118.8, 122.2, 127.8, 128.5, 131.2, 131.8, 137.6, 144.9, 145.9, 152.8, 157.9, 166.2; Anal. for $C_{42}H_{34}N_8O_5$ (730.7) Calcd: C, 69.03; H, 4.69; N, 15.33; found: C, 69.19; H, 4.54; N, 15.17%.

General procedure for the synthesis of bis(thieno[2,3-b] pyridines) 20

Method 'A' A mixture of bis(pyridine-2(1*H*)-thione) **5** (5 mmol) and each of chloroacetone **19a**, chloroacetonitrile **19b**, chloroacetamide **19c**, ethyl chloroacetate **19d**, 2-bromo-1-phenylethanone **19e** or 2-bromo-1-(4-chlorophenyl)ethan-1-one **19f** (10 mmol) in ethanolic sodium ethoxide solution (prepared by dissolving 22 mmol of sodium in 15 mL of ethanol) was heated at reflux for 4 h. The product was collected by filtration, washed with water, ethanol, dried and recrystallized from the appropriate solvent.

Method 'B' A mixture of bis(nicotinonitrile) **7** (5 mmol) in ethanolic sodium ethoxide solution (prepared by dissolving 11 mmol of sodium in 15 mL of ethanol) was heated at reflux for 2 h. The product was collected by filtration, washed with water, ethanol, dried and recrystallized from dioxane to yield **20a**.

6,6'-(Oxybis(4,1-phenylene))bis(2-acetyl-3-aminothieno[2,3-b]pyridine) (20a)

Orange solid (dioxane/ethanol mixture; 82% using Method 'A'; 70% using Method 'B'); m.p. 232 °C; IR (υ cm^{-1}): 3465, 3292 (NH_2), 1629 (CO); ^{1}H-NMR (DMSO-d_6): δ 2.39 (s, 6H, 2 CH_3), 6.88 (s, 4H, 2 NH_2), 7.10 (d, J = 8.8 Hz, 4H, ArH), 7.98 (d, J = 5.6 Hz, 2H, 2 pyridine-H5), 8.17 (d, J = 8.8 Hz, 4H, ArH), 8.47 (d, J = 5.6 Hz, 2H, 2 pyridine-H4); ^{13}C-NMR (DMSO-d_6): δ 29.5, 114.9, 116.1, 117.2, 124.0, 128.9, 130.4, 131.6, 149.5, 157.2, 158.8, 161.3, 192.1; MS *m/z* (%): 550 (M^+, 27.1); Anal. for $C_{30}H_{22}N_4O_3S_2$ Calcd: C, 65.44; H, 4.03; N, 10.17; found: C, 65.67; H, 4.19; N, 10.00%.

6,6'-(Oxybis(4,1-phenylene))bis(3-aminothieno[2,3-b]pyridine-2-carbonitrile) (20b)

Yellow solid (dioxane, 84%); m.p. 266–269 °C; IR (υ cm^{-1}): 3469, 3228 (NH_2), 2199 (CN); ^{1}H-NMR (DMSO-d_6): δ 5.99 (s, 4H, 2 NH_2), 7.08 (d, J = 8.8 Hz, 4H, ArH), 8.07 (d, J = 5.2 Hz, 2H, 2 pyridine-H5), 8.15 (d, J = 8.8 Hz, 4H, ArH), 8.54 (d, J = 5.2 Hz, 2H, 2 pyridine-H4); ^{13}C-NMR (DMSO-d_6): δ 71.7, 114.8, 116.0, 116.3, 122.7, 128.7, 130.2, 131.2, 150.8, 157.5, 159.1, 161.4; MS *m/z* (%): 516 (M^+, 52.1); Anal. for $C_{28}H_{16}N_6OS_2$ Calcd: C, 65.10; H, 3.12; N, 16.27; found: C, 65.33; H, 3.28; N, 16.02%.

6,6'-(Oxybis(4,1-phenylene))bis(3-aminothieno[2,3-b]pyridine-2-carboxamide) (20c)

Yellow solid (dioxane, 80%); m.p. 268 °C; IR (υ cm^{-1}): 3468, 3313, 3255 (2 NH_2), 1658 (CO); ^{1}H-NMR (DMSO-d_6): δ 6.23 (s, 4H, 2 NH_2), 7.09 (d, J = 8.8 Hz, 4H, ArH), 7.22, 7.33 (2 br s, 4H, 2 $CONH_2$), 7.99 (d, J = 5.6 Hz, 2H, 2 pyridine-H5), 8.14 (d, J = 8.8 Hz, 4H, ArH), 8.47 (d, J = 5.6 Hz, 2H, 2 pyridine-H4); ^{13}C-NMR (DMSO-d_6): δ 97.2, 114.9, 115.6, 124.5, 128.9, 130.3, 131.7, 145.7, 156.0, 158.6, 160.7, 167.4; Anal. for $C_{28}H_{20}N_6O_3S_2$ (552.6) Calcd: C, 60.86; H, 3.65; N, 15.21; found: C, 60.59; H, 3.74; N, 14.99%.

Diethyl 6,6'-(oxybis(4,1-phenylene))bis(3-aminothieno[2,3-b]pyridine-2-carboxylate) (20d)

Yellow solid (dioxane, 82%); m.p. 258 °C; IR (υ cm^{-1}): 3464, 3332 (NH_2), 1666 (CO); ^{1}H-NMR (DMSO-d_6): δ 1.30 (t, J = 7.2 Hz, 6H, 2 OCH_2CH_3), 4.29 (q, J = 7.2 Hz, 4H, 2 OCH_2CH_3), 6.14 (s, 4H, 2 NH_2), 7.09 (d, J = 8.8 Hz, 4H, ArH), 7.95 (d, J = 5.6 Hz, 2H, 2 pyridine-H5), 8.18 (d, J = 8.8 Hz, 4H, ArH), 8.43 (d, J = 5.6 Hz, 2H, 2 pyridine-H4); ^{13}C-NMR (DMSO-d_6): δ 13.9, 61.2, 114.8, 116.0, 116.9, 124.4, 128.7, 130.7, 131.2, 148.5, 157.5, 159.2, 161.1, 166.2; Anal. for $C_{32}H_{26}N_4O_5S_2$ (610.7) Calcd: C, 62.94; H, 4.29; N, 9.17; found: C, 63.22; H, 4.04; N, 9.35%.

6,6'-(Oxybis(4,1-phenylene))bis(3-amino-2-benzoylthieno-[2,3-b]pyridine) (20e)

Yellow solid (dioxane/ethanol mixture, 77%); m.p. 264–265 °C; IR (υ cm^{-1}): 3465, 3272 (NH_2); ^{1}H-NMR (DMSO-d_6): δ 7.11 (d, J = 8.8 Hz, 4H, ArH), 7.25 (br s, 4H, 2 NH_2), 7.53 (t, J = 7.2 Hz, 2H, ArH), 7.63 (t, J = 7.2 Hz, 4H, ArH), 7.79 (d, J = 7.2 Hz, 4H, ArH), 7.97 (d, J = 5.2 Hz, 2H, 2 pyridine-H5), 8.18 (d, J = 8.8 Hz, 4H, Ar–H's), 8.49 (d, J = 5.2 Hz, 2H, 2 pyridine-H4); ^{13}C-NMR (DMSO-d_6): δ 115.2, 116.3, 116.9, 124.2, 127.8, 128.6, 128.8, 130.7, 131.5, 132.2, 141.6, 147.3, 156.6, 158.5, 161.2, 189.5; Anal. for $C_{40}H_{26}N_4O_3S_2$ (674.7) Calcd: C, 71.20; H, 3.88; N, 8.30; found: C, 71.39; H, 4.12; N, 8.12%.

6,6'-(Oxybis(4,1-phenylene))bis(3-amino-2-(4-chlorobenzoyl)thieno[2,3-b]pyridine) (20f)

Yellow solid (dioxane, 79%); m.p. 274–277 °C; IR (υ cm^{-1}): 3468, 3272 (NH_2); ^{1}H-NMR (DMSO-d_6): δ 7.10 (d, J = 8.8 Hz, 4H, ArH), 7.28 (br s, 4H, 2 NH_2), 7.63 (d, J = 7.2 Hz, 4H, ArH), 7.83 (d, J = 7.2 Hz, 4H, ArH), 7.95 (d, J = 5.2 Hz, 2H, 2 pyridine-H5), 8.17 (d, J = 8.8 Hz, 4H, ArH), 8.51 (d, J = 5.2 Hz, 2H, 2 pyridine-H4); ^{13}C-NMR (DMSO-d_6): δ 115.0, 116.3, 116.5, 124.0, 128.5, 129.8, 130.5, 130.7, 131.8, 136.2, 139.6, 145.9, 156.9, 158.3, 161.5, 187.6; Anal. for $C_{40}H_{24}Cl_2N_4O_3S_2$ (743.6) Calcd: C, 64.60; H, 3.25; N, 7.53; found: C, 64.86; H, 3.41; N, 7.39%.

AChE inhibition assay

AChE inhibitory activities were assessed using Ellman procedure [44, 56] with minor changes. Stock solutions of the tested hybrids were prepared in a mixture of 1 mL DMSO and 9 mL methanol, followed by dilution in the buffer KH_2PO_4/K_2HPO_4 (50 mM, pH 7.7) to acquire the final concentration. To 60 µL of 50 mM phosphate buffer (pH 7.7), 10 µL of the respective assayed sample (at stock solution of 0.5 mM) was applied. Then, 10 µL of 0.005 unit per well enzyme solution (AChE, E.C.3.1.1.7, Type V-S, lyophilized powder, from *electric eel*, 1000 units, Sigma-Aldrich) was applied. The resulting content was mixed and pre-read at 405 nm, then incubated at 37 °C for 10 min. In each well, the reaction was started by adding 10 µL of acetylthiocholine iodide (0.5 mM), followed by adding 10 µL of 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB, 0.5 mM per well). At 37 °C, the wells were incubated, then the absorption of each well was measured at 405 nm using the 96-well plate reader Synergy HT, Biotek, USA. Eserine (0.5 mM) was used as a positive control. The inhibition percentages are calculated by the following formula:

$$\text{Inhibition percentage} = \left(\text{Control}_{\text{absorbance}} - \text{Sample}_{\text{absorbance}}\right) \times 100/\text{Control}_{\text{absorbance}}$$

Each compound was tested in triplicates at concentration of 100 µM.

DPPH radical-scavenging assay

DPPH radical-scavenging activity was assessed according to Thuong et al. procedure with minor changes [45, 68]. Methanol was used as a solvent to assess the tested derivatives. 1 mL of methanolic DPPH solution (0.3 mM) was applied to 2.5 mL of the tested derivative in 96-well plates. 1 mL of methanol was applied, and the solution was mixed for a minute at rt and incubated in a dark place. After 30 min, the absorbance of the reaction mixture was calculated at 520 nm on a microplate reader. The reading blank consisted of 2.5 mL of estimated hybrid and 1 mL of methanol, meanwhile the mixture of 1 mL DPPH and 2.5 mL of methanol was used as negative control. The percent of the antioxidant activity is calculated using the equation:

$$\text{Inhibition percentage} = \left(\text{Control}_{\text{absorbance}} - \text{Sample}_{\text{absorbance}}\right) \times 100/\text{Control}_{\text{absorbance}}$$

The new hybrids, as well as the reference ascorbic acid, have been examined in triplicates at concentration of 25 µg/mL.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13738-022-02614-8.

References

1. E.D. AlFadly, P.A. Elzahhar, A. Tramarin, S. Elkazaz, H. Shaltout, M.M. Abu-Serie, J. Janockova, O. Soukup, D.A. Ghareeb, A.F. El-Yazbi, R.W. Rafeh, Eur. J. Med. Chem. **167**, 161–186 (2019). https://doi.org/10.1016/j.ejmech.2019.02.012
2. M. Saxena, R. Dubey, Curr. Top. Med. Chem. **19**, 264–275 (2019). https://doi.org/10.2174/1568026619666190128125912
3. W. Liu, M. Lang, M.B. Youdim, T. Amit, Y. Sun, Z. Zhang, Y. Wang, O. Weinreb, Neuropharmacology **109**, 376–385 (2016). https://doi.org/10.1016/j.neuropharm.2016.06.013
4. A. Kumar, A. Singh, Pharmacol. Rep. **67**, 195–203 (2015). https://doi.org/10.1016/j.pharep.2014.09.004
5. A.S. Gurjar, M.N. Darekar, K.Y. Yeong, L. Ooi, Bioorg. Med. Chem. **26**, 1511–1522 (2018). https://doi.org/10.1016/j.bmc.2018.01.029
6. J. Trujillo-Ferrara, L. Montoya Cano, M. Espinoza-Fonseca, Bioorg. Med. Chem. Lett. **13**, 1825–1827 (2003). https://doi.org/10.1016/S0960-894X(03)00198-7
7. F. Vafadarnejad, M. Mahdavi, E. Karimpour-Razkenari, N. Edraki, B. Sameem, M. Khanavi, M. Saeedi, T. Akbarzadeh, Bioorg. Chem. **77**, 311–319 (2018). https://doi.org/10.1016/j.bioorg.2018.01.013
8. J. Joubert, G.B. Foka, B.P. Repsold, D.W. Oliver, E. Kapp, S.F. Malan, Eur. J. Med. Chem. **125**, 853–864 (2017). https://doi.org/10.1016/j.ejmech.2016.09.041
9. M.T. Heneka, M.J. Carson, J. El Khoury, G.E. Landreth, F. Brosseron, D.L. Feinstein, A.H. Jacobs, T. Wyss-Coray, J. Vitorica, R.M. Ransohoff, K. Herrup, Lancet Neurol. **14**, 388–405 (2015). https://doi.org/10.1016/S1474-4422(15)70016-5
10. C.K. Glass, K. Saijo, B. Winner, M.C. Marchetto, F.H. Gage, Cell **140**, 918–934 (2010). https://doi.org/10.1016/j.cell.2010.02.016
11. J. Hardy, J. Neurochem. **110**, 1129–1134 (2009). https://doi.org/10.1111/j.1471-4159.2009.06181.x
12. M. Weinstock, E. Groner, Chem. Biol. Interact. **175**, 216–221 (2008). https://doi.org/10.1016/j.cbi.2008.03.014
13. J.L. Cummings, R. Doody, C. Clark, Neurology **69**, 1622–1634 (2007). https://doi.org/10.1212/01.wnl.0000295996.54210.69
14. J. Rodda, J. Carter, BMJ **344**, e2986 (2012). https://doi.org/10.1136/bmj.e2986
15. A. Więckowska, M. Kołaczkowski, A. Bucki, J. Godyń, M. Marcinkowska, K. Więckowski, P. Zaręba, A. Siwek, G. Kazek, M. Głuch-Lutwin, P. Mierzejewski, Eur. J. Med. Chem. **124**, 63–81 (2016). https://doi.org/10.1016/j.ejmech.2016.08.016
16. M.B. Colovic, D.Z. Krstic, T.D. Lazarevic-Pasti, A.M. Bondzic, V.M. Vasic, Curr. Neuropharmacol. **11**, 315–335 (2013). https://doi.org/10.2174/1570159X11311030006
17. M. Mroueh, W.H. Faour, W.N. Shebaby, C.F. Daher, T.M. Ibrahim, H.M. Ragab, Bioorg. Chem. **100**, 103895 (2020). https://doi.org/10.1016/j.bioorg.2020.103895
18. T. Umar, S. Shalini, M.K. Raza, S. Gusain, J. Kumar, P. Seth, M. Tiwari, N. Hoda, Eur. J. Med. Chem. **175**, 2–19 (2019). https://doi.org/10.1016/j.ejmech.2019.04.038
19. M. do Carmo Carreiras, J. Marco-Contelles, Synlett **32**, 1987–2012 (2021). https://doi.org/10.1055/s-0040-1719823
20. M. Saeedi, M. Safavi, E. Allahabadi, A. Rastegari, R. Hariri, S. Jafari, S.N. Bukhari, S.S. Mirfazli, O. Firuzi, N. Edraki, M. Mahdavi, Arch. Pharm. **353**, 2000101 (2020). https://doi.org/10.1002/ardp.202000101
21. B. Zhao, Y. Li, P. Xu, Y. Dai, C. Luo, Y. Sun, J. Ai, M. Geng, W. Duan, A.C.S. Med, Chem. Lett. **7**, 629–634 (2016). https://doi.org/10.1021/acsmedchemlett.6b00066
22. R.F. Barghash, W.M. Eldehna, M. Kovalová, V. Vojáčková, V. Kryštof, H.A. Abdel-Aziz, Eur. J. Med. Chem. **227**, 113952 (2022). https://doi.org/10.1016/j.ejmech.2021.113952
23. P. Nagender, G.M. Reddy, R.N. Kumar, Y. Poornachandra, C.G. Kumar, B. Narsaiah, Bioorg. Med. Chem. Lett. **24**, 2905–2908 (2014). https://doi.org/10.1016/j.bmcl.2014.04.084
24. M.A. El-Borai, H.F. Rizk, M.F. Abd-Aal, I.Y. El-Deeb, Eur. J. Med. Chem. **48**, 92–96 (2012). https://doi.org/10.1016/j.ejmech.2011.11.038
25. A.M.R. Bernardino, A.R. de Azevedo, L.C. da Silva Pinheiro, J.C. Borges, V.L. Carvalho, M.D. Miranda, M.D.F. de Meneses, M. Nascimento, D. Ferreira, M.A. Rebello, V.A.G.G. Da Silva, Med. Chem. Res. **16**, 352–369 (2007). https://doi.org/10.1007/s00044-007-9035-6
26. A.C. Medeiros, J.C. Borges, K.M. Becker, R.F. Rodrigues, L.L. Leon, M. Canto-Cavalheiro, A.M. Bernardino, M.C.D. Souza, L.F. Pedrosa, J. Braz. Chem. Soc. **29**, 159–167 (2018). https://doi.org/10.21577/0103-5053.20170126
27. S.M.H. Sanad, A.E.M. Mekky, Chem. Biodivers. **19**, e202100500 (2022). https://doi.org/10.1002/cbdv.202100500
28. I.H. Eissa, A.M. El-Naggar, M.A. El-Hashash, Bioorg. Chem. **67**, 43–56 (2016). https://doi.org/10.1016/j.bioorg.2016.05.006
29. C.M.S. Menezes, C.M.R. Sant'Anna, C.R. Rodrigues, E.J. Barreiro, J. Mol. Struct. **579**, 31–39 (2002). https://doi.org/10.1016/S0166-1280(01)00677-7

30. S.M. Emam, F.A. El-Saied, S.A. Abou El-Enein, H.A. El-Shater, Spectrochim. Acta Part A **72**, 291–297 (2009). https://doi.org/10.1016/j.saa.2008.09.015
31. J. Quiroga, Y. Villarreal, J. Gálvez, A. Ortíz, B. Insuasty, R. Abonia, M. Raimondi, S. Zacchino, Chem. Pharm. Bull. **65**, 143–150 (2017). https://doi.org/10.1248/cpb.c16-00652
32. P.K. Sharma, K. Singh, S. Kumar, P. Kumar, S.N. Dhawan, S. Lal, H. Ulbrich, G. Dannhardt, Med. Chem. Res. **20**, 239–244 (2011). https://doi.org/10.1007/s00044-010-9312-7
33. S.M.H. Sanad, A.E.M. Mekky, J. Iran. Chem. Soc. **17**, 3299–3315 (2020). https://doi.org/10.1007/s13738-020-01987-y
34. D. Shuck-Lee, F.F. Chen, R. Willard, S. Raman, R. Ptak, M.L. Hammarskjold, D. Rekosh, Antimicrob. Agents Chemother. **52**, 3169–3179 (2008). https://doi.org/10.1128/AAC.00274-08
35. K. Madhusudana, B. Shireesha, V.G.M. Naidu, S. Ramakrishna, B. Narsaiah, A.R. Rao, P.V. Diwan, Eur. J. Pharmacol. **678**, 48–54 (2012). https://doi.org/10.1016/j.ejphar.2011.12.019
36. M. Kamata, T. Yamashita, A. Kina, M. Funata, A. Mizukami, M. Sasaki, A. Tani, M. Funami, N. Amano, K. Fukatsu, Bioorg. Med. Chem. Lett. **22**, 3643–3647 (2012). https://doi.org/10.1016/j.bmcl.2012.04.047
37. E.M. Mohi El-Deen, A. El-Meguid, A. Eman, S. Hasabelnaby, E.A. Karam, E.S. Nossier, Molecules **24**, 3650–3669 (2019). https://doi.org/10.3390/molecules24203650
38. M.S. Mohamed, Y.E. Mansour, H.K. Amin, M.E. El-Araby, J. Enzyme Inhibit. Med. Chem. **33**, 755–767 (2018). https://doi.org/10.1080/14756366.2018.1457657
39. C. Karthikeyan, R. Malla, C.R. Ashby Jr., H. Amawi, K.L. Abbott, J. Moore, J. Chen, C. Balch, C. Lee, P.C. Flannery, P. Trivedi, Cancer Lett. **376**, 118–126 (2016). https://doi.org/10.1016/j.canlet.2016.03.030
40. S.M.H. Sanad, A.E.M. Mekky, ChemistrySelect **5**, 8494–8503 (2020). https://doi.org/10.1002/slct.202001208
41. A. Daina, O. Michielin, V.A. Zoete, Sci. Rep. **7**, 42717 (2017). https://doi.org/10.1038/srep42717
42. A.A.M. Ahmed, A.E.M. Mekky, S.M.H. Sanad, Synth. Commun. **52**, 912–925 (2022). https://doi.org/10.1080/00397911.2022.2056853
43. A.E.M. Mekky, S.M.H. Sanad, T.T. El-Idreesy, Synth. Commun. **51**, 3332–3344 (2021). https://doi.org/10.1080/00397911.2021.1970774
44. A.E.M. Mekky, S.M.H. Sanad, J. Heterocyclic Chem. **57**, 4278–4290 (2020). https://doi.org/10.1002/jhet.4134
45. S.M.H. Sanad, A.E.M. Mekky, J. Iran. Chem. Soc. **18**, 213–224 (2021). https://doi.org/10.1007/s13738-020-02018-6
46. A. Tlili, F. Monnier, M. Taillefer, Chem. Eur. J. **16**, 12299–12302 (2010). https://doi.org/10.1002/chem.201001373
47. A.E.M. Mekky, A.S. Al-Bogami, J. Heterocycl. Chem. **53**, 595–605 (2016). https://doi.org/10.1002/jhet.2328
48. A.S. Al-Bogami, A.E.M. Mekky, J. Heterocycl. Chem. **53**, 1554–1562 (2016). https://doi.org/10.1002/jhet.2462
49. B. Hu, P. Zhou, Q. Zhang, Y. Wang, S. Zhao, L. Lu, S. Yan, F. Yu, J. Org, Chem. **83**, 14978–14986 (2018). https://doi.org/10.1021/acs.joc.8b02235
50. Q. Chen, X. Wanga, C. Wena, Y. Huanga, X. Yana, J. Zeng, RSC Adv. **7**, 39758–39761 (2017). https://doi.org/10.1039/C7RA06904A
51. A.S. Kulikov, M.A. Epishina, E.S. Zhilin, A.D. Shuvaev, L.L. Fershtat, N.N. Makhova, Mendeleev Commun. **31**, 42–45 (2021). https://doi.org/10.1016/j.mencom.2021.01.012
52. A.E.M. Mekky, S.M.H. Sanad, Polycyclic Aromat. Compd. **41**, 936–949 (2021). https://doi.org/10.1080/10406638.2019.1631194
53. C.M. Holden, M.F. Greaney, Chem. Eur. J. **23**, 8992–9008 (2017). https://doi.org/10.1002/chem.201700353
54. S.M.H. Sanad, A.E.M. Mekky, J. Heterocycl. Chem. **57**, 3142–3152 (2020). https://doi.org/10.1002/jhet.4021
55. S.M.H. Sanad, A.M. Abdel Fattah, F.A. Attaby, M.A.A. Elneairy, Can. J. Chem. **97**, 53–60 (2019). https://doi.org/10.1139/cjc-2017-0721
56. G.L. Ellman, K.D. Courtney, V. Andres Jr., R.M. Featherstone, Biochem. Pharm. **7**, 88–90 (1961). https://doi.org/10.1016/0006-2952(61)90145-9
57. M. Alipour, M. Khoobi, A. Foroumadi, H. Nadri, A. Moradi, A. Sakhteman, M. Ghandi, A. Shafiee, Bioorg. Med. Chem. **20**, 7214–7222 (2020). https://doi.org/10.1016/j.bmc.2012.08.052
58. T. Pan, S. Xie, Y. Zhou, J. Hu, H. Luo, X. Li, L. Huang, Bioorg. Med. Chem. Lett. **29**, 2150–2152 (2019). https://doi.org/10.1016/j.bmcl.2019.06.056
59. D. Silva, M. Chioua, A. Samadi, M.C. Carreiras, M.L. Jimeno, E. Mendes, C. de Los Ríos, A. Romero, M. Villarroya, M.G. López, J. Marco-Contelles, Eur. J. Med. Chem. **46**, 4676–4681 (2011). https://doi.org/10.1016/j.ejmech.2011.05.068
60. E.J. Barreiro, C.A. Camara, H. Verli, L. Brazil-Más, N.G. Castro, W.M. Cintra, Y. Aracava, C.R. Rodrigues, C.A. Fraga, J. Med. Chem. **46**, 1144–1152 (2003). https://doi.org/10.1021/jm020391n
61. F.A. Attaby, A.M. Abdel-Fattah, L.M. Shaif, M.M. Elsayed, Phosphorus Sulfur Silicon Relat. Elem. **185**, 129–139 (2009). https://doi.org/10.1080/10426500902717333
62. F.A. Attaby, A.M. Abdel-Fattah, L.M. Shaif, M.M. Elsayed, Phosphorus Sulfur Silicon Relat. Elem. **185**, 668–679 (2010). https://doi.org/10.1080/10426500902917644
63. Y. Feng, X. Wang, Oxid. Med. Cell Longev. **2012**, 472932 (2012). https://doi.org/10.1155/2012/472932
64. M.E. McLellan, S.T. Kajdasz, B.T. Hyman, B.J. Bacskai, J. Neurosci. **23**, 2212–2217 (2003). https://doi.org/10.1523/JNEUROSCI.23-06-02212.2003
65. S. Çakmak, S. Kansiz, M. Azam, C.C. Ersanlı, O. Idil, A. Veyisoğlu, H. Yakan, H. Kütük, A. Chutia, ACS Omega **7**, 11320–11329 (2022). https://doi.org/10.1021/acsomega.2c00318
66. J.A. Arnott, S.L. Planey, Expert Opin. Drug Discov. **7**, 863–875 (2012). https://doi.org/10.1517/17460441.2012.714363
67. C.A. Lipinski, Drug Discov. Today Technol. **1**, 337–341 (2004). https://doi.org/10.1016/j.ddtec.2004.11.007
68. P.T. Thuong, T.M. Hung, T.M. Ngoc, D.T. Ha, B.S. Min, S.J. Kwack, T.S. Kang, J.S. Choi, K. Bae, Phytother. Res. **24**, 101–106 (2010). https://doi.org/10.1002/ptr.2890

Authors and Affiliations

Ahmed A. M. Ahmed[1,2] · Ahmed E. M. Mekky[1] · Sherif M. H. Sanad[1]

[1] Chemistry Department, Faculty of Science, Cairo University, Giza, Egypt

[2] Common First Year Deanship, Jouf University, Sakaka, Kingdom of Saudi Arabia