Received: 3 September 2020 | Revised: 1 January 2021 | Accepted: 4 January 2021

DOI: 10.1002/ardp.202000330

FULL PAPER

ARCH PHARM DPhG
Archiv der Pharmazie

Synthesis of *N*-alkylated pyrazolo[3,4-*d*]pyrimidine analogs and evaluation of acetylcholinesterase and carbonic anhydrase inhibition properties

Busra O. Aydin[1] | Derya Anil[1,2] | Yeliz Demir[3]

[1]Department of Chemistry, Faculty of Science, Ataturk University, Erzurum, Turkey

[2]Department of Chemistry and Chemical Process Technologies, Technical Sciences Vocational School, Ataturk University, Erzurum, Turkey

[3]Department of Pharmacy Services, Nihat Delibalta Göle Vocational School, Ardahan University, Ardahan, Turkey

Correspondence
Derya Anil, Technical Sciences Vocational School, Ataturk University, Erzurum 25240, Turkey.
Email: derya.anil@atauni.edu.tr and daktas61@gmail.com

Abstract

Fused pyrimidines, especially pyrazolo[3,4-*d*]pyrimidines, are among the most preferred building blocks for pharmacology studies, as they exhibit a broad spectrum of biological activity. In this study, new derivatives of pyrazolo[3,4-*d*]pyrimidine were synthesized by alkylation of the N1 nitrogen atom. We synthesized 3-iodo-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **2** from commercially available aminopyrazolopyrimidine **1** using *N*-iodosuccinimide as an iodinating agent. The synthesis of compound **2** started with nucleophilic substitution of 3-iodo-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine with R–X (X: –OMs, –Br, –Cl), affording *N*-alkylated pyrazolo[3,4-*d*]pyrimidine. We performed this synthesis using a weak inorganic base and the mild temperature was also used for a two-step procedure to generate *N*-alkylated pyrazolo[3,4-*d*]pyrimidine derivatives. Also, all compounds were tested for their ability to inhibit acetylcholinesterase (AChE) and the human carbonic anhydrase (hCA) isoforms I and II, with K_i values in the range of 15.41 ± 1.39–63.03 ± 10.68 nM for AChE, 17.68 ± 1.92–66.27 ± 5.43 nM for hCA I, and 8.41 ± 2.03–28.60 ± 7.32 nM for hCA II. Notably, compound **10** was the most selective and potent CA I inhibitor with a significant selectivity ratio of 26.90.

KEYWORDS
acetylcholinesterase, carbonic anhydrase, enzyme inhibition, pyrazolopyrimidine

1 | INTRODUCTION

Heterocyclic compounds have a broad range of applications, in medicinal chemistry, agrochemicals, polymers, and products from various industries.[1] More specifically, nitrogen-containing heterocycles are plentiful in nature, existing in many natural products such as alkaloids, vitamins, antibiotics, and hormones.[2] Approximately 60% of FDA-approved drugs contain heterocyclic nitrogen in their structure. The pyrimidine nucleus is a significant class of heterocyclic compounds for their medicinal properties due to the presence of pyrimidine base in uracil, cytosine, and thymine, which form the building blocks of DNA and RNA.[3]

Pyrimidine and its fused analogs have attracted considerable attention due to various biological activities. Among fused pyrimidines, pyrazolopyrimidine is a significant scaffold present in numerous biologically active compounds. As pyrazolopyrimidines have a structural resemblance with purines, they are considered biologically active isomeric purine analogs present in DNA and RNA.[4] Pyrazolo[3,4-*d*]pyrimidines are reported to have various pharmacological properties as antiviral, anticoagulant, antimicrobial, antitumor, analgesic, and antileukemic agent.[5] Recently, the pyrazolo[3,4-*d*]pyrimidine ring system has been a significant pharmacophore for anticancer drug discovery.[6–8] Pyrazolo[3,4-*d*]pyrimidines are potent and selective inhibitors of many kinases, which have a key role in cancer cell proliferation.[9–11]

The pyrazolopyrimidine nucleus has been the core of synthetic drug molecules and is still a frequently occurring motif in many biologically active compounds (Figure 1). For example, allopurinol,

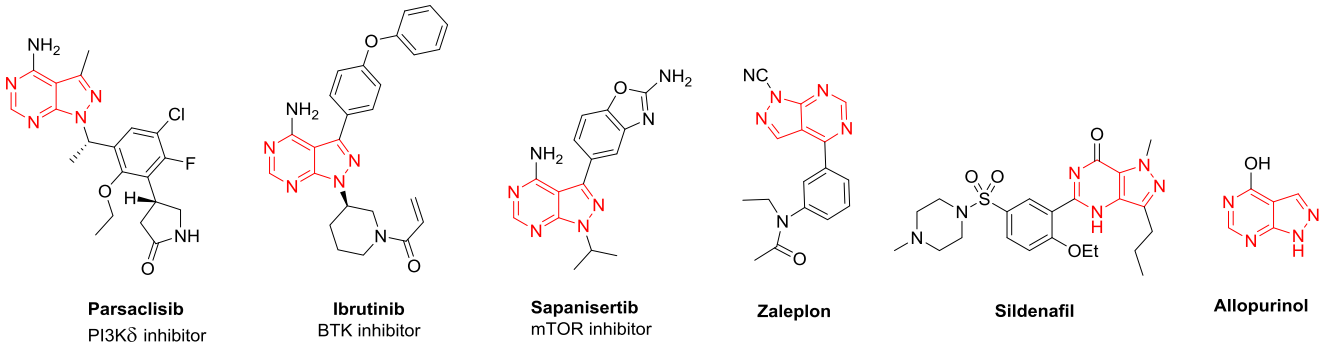


FIGURE 1 Clinically approved drugs containing the pyrazolopyrimidine ring

ibrutinib, and parsaclisib have pyrazolopyrimidine core structures containing anticancer agents currently approved by the Food and Drug Administration (FDA). Sapanisertib is an experimental kinase inhibitor, which is currently in clinical trials for several cancer indications.[12] Zaleplon and sildenafil are other FDA-approved drugs containing the pyrazolopyrimidine core structure.[13]

The carbonic anhydrases (CAs) play a role in the reversible hydration of CO_2 into bicarbonate and protons.[14,15] They also play a key role in numerous pathological and biological processes such as tumor growth, respiration, secretion of electrolytes in various tissues and organs, homeostasis of pH, biosynthetic reactions (i.e., ureagenesis and lipogenesis), bone resorption, and calcification.[16–20] Hence, CA isoforms play a crucial role as therapeutic goals; their inhibitors have a significant task for pharmacological areas with a wide range of ailments such as cancer. osteoporosis, glaucoma, edema, epilepsy, and obesity.[21,22] Inhibitors of cytosolic human carbonic anhydrase (hCA) isoforms I and II are mainly employed as antiepileptic drugs, antiedema drugs, antiglaucoma drugs, and diuretics.[23,24]

Alzheimer's disease (AD) is a neuropathological disorder, which mostly affects older people.[25] AD may comprise thinking or problem-solving, memory loss, and difficulties with language. AD is characterized by multiple cortical disturbances, such as intellectual function disorders, inability to perform daily life activity, decline of learning skills, and loss of memory.[26,27] There are various hypotheses put forward to explain the AD. Among them, the cholinergic hypothesis (covering butyrylcholinesterase or BuChE enzymes and acetylcholinesterase or AChE) is the most considerable hypothesis for the treatment of AD.[28] AChE is primarily responsible for the hydrolysis of acetylcholine into acetate and choline.[29] Inhibition of the AChE enzyme gives rise to the enhancement of cognitive abilities such as memory functions, attention span, and language skills in AD.[30,31] Hence, the development of potent AChE inhibitors may be an essential approach for AD treatment.

In the literature, pyrimidine analogs have been studied as AChE and CAs inhibitors. For instance, Zhi et al.[32] designed and synthesized some 6-acetyl-5*H*-thiazolo[3,2-*a*]pyrimidine derivatives and studied their in vitro AChE inhibitory activity. Kuday et al. [33] synthesized 11 new pyrido[2,3-*d*]pyrimidine derivatives containing indole rings and assayed their effects on hCA I and II. They also examined a structure–activity relationship. Sharma and Shukla[34] analyzed the in silico molecular docking of six pyrimidine derivatives with EFGR and CA IX, proposing their role as inhibitors in cancer studies. They tested these compounds in silico for drug-likeness and anticancer activity by docking with the protein via pyrx docking software.

According to the above information, in this study, we worked on the synthesis of 13 novel *N*-alkylated pyrazolo[3,4-*d*]pyrimidine derivatives. Also, we studied their in vitro biological effects on AChE and hCA I and II isoforms.

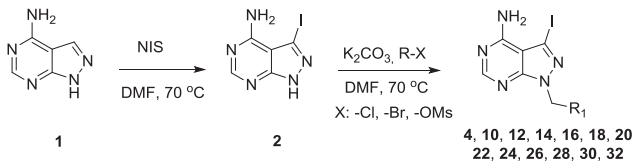
2 | RESULTS AND DISCUSSION

2.1 | Chemistry

The synthesis of *N*-alkylated pyrazolo[3,4-*d*]pyrimidine derivatives is an important research area in the literature.[35–38] Minor structural modifications, which are sometimes single-atom or group changes, can have a dramatic impact on the activities of the compounds. In this study, we report the synthesis of *N*-alkylated 3-iodo-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine derivatives. *N*-Alkylation was performed using alkyl or benzyl derivatives under basic conditions.

The alkylation of 1*H*-pyrazolo[3,4-*d*]pyrimidines has typically been observed to be selective for N1 under various conditions such as phase-transfer catalysis in benzene, Cs_2CO_3 in dimethylformamide (DMF), strong base, Mitsunobu reaction, or weak base under high-temperature conditions.[39,40] We developed useful synthetic methodologies for the synthesis of new *N*-alkylated pyrazolo[3,4-*d*]pyrimidine derivatives with the use of inorganic weak base at 70°C. All synthesized compounds contain iodine atoms in their structure and they can be easily functionalized by the coupling reaction.

The general method used for the *N*-alkylation of 1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine is shown in Scheme 1. First, a commercially available



SCHEME 1 General synthesis of *N*-alkylated pyrazolopyrimidines

4-amino-1*H*-pyrazolo[3,4-*d*]pyrimidine (**1**) compound was treated with *N*-iodosuccinimide in DMF at 70°C to afford compound **2**.[41] We aimed for alkylation with derivatives that have a simple structure and different electronic properties (halogen-containing benzyl, ethyne, alkyne, or propyl). The *N*-alkylation of compound 3-iodo-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (**2**) was performed using R–X (X: –Cl, –Br, –OMs) derivatives in the presence of potassium carbonate in DMF.

First, we started with chloroalkyl derivatives. Compound **2** was made to react with benzyl chloride in the presence of potassium carbonate in DMF and heated at 70°C. Benzyl-alkylated product **4** was obtained with a 44% yield. To obtain epoxy and hydroxy alkylation products (**6** and **8**), a reaction was carried out under the same conditions using epichlorohydrin (**5**) and 2-chloroethanol (**7**), but desired products could not be generated (Table 1).

The alkylation reaction with alkyl bromide, benzyl bromide, and cinnamyl bromide reagents yielded compounds **10**, **12**, **14**, and **16**, as shown in Table 2. Treatment of compound **2** with **9**, **11**, **13**, and **15** and potassium carbonate in dry DMF afforded a series of N-substituted pyrazolo[3,4-*d*]pyrimidine derivatives that include **10**, **12**, **14**, and **16** with good-to-excellent yield. Compound **16** was synthesized using the alkyl halide mentioned in the literature with a 73% yield using high equivalent K_2CO_3 at 60°C.[42] We obtained compound **16** with 87% yield using low equivalent K_2CO_3 at 70°C. Here, compound **10** was obtained using 4-bromobenzyl bromide in a reaction. Unlike other derivatives, 4-bromobenzyl was found to be attached to both C3 and N1 positions. This was evidenced by all spectral data (^{1}H nuclear magnetic resonance [NMR], ^{13}C NMR, high-resolution mass spectrometry [HRMS]). Reaction yield rates were excellent, as seen in Table 2.

In the next step of our study, alkylation was performed using **17**, **19**, **21**, **23**, **25**, **27**, **29**, and **31**. The OH group in the structure of the reagents was converted to an easily purified mesylate with MsCl and NEt_3. The *N*-alkylation reaction previously performed with alkyl bromine and chlorine reagents was carried out under the same reaction conditions using reagents containing the –OMs functional group. Compound **20** was synthesized according to a procedure in the literature, using alkyl bromide and higher equivalent K_2CO_3 at 130°C, and it resulted in a 42% yield.[38] We obtained compound **20** using phenethyl methanesulfonate with 66% yield using low equivalent K_2CO_3 at 70°C. Also, compound **18** was synthesized according to a procedure in the literature, but it yielded different results.[43] The compound was an intermediate, so results were not clear enough to be compared. Compounds **29** and **31** contain OH groups in the aromatic ring. As aromatic OH groups have acidic properties, an alkylation step was performed in these groups after being converted to an –OMs derivative. Obtained –OMs intermediates (**17**, **19**, **21**, **23**, **25**, **27**, **29**, and **31**) were used for the N1

TABLE 1 *N*-Alkylated pyrazolopyrimidines from alkyl chloride

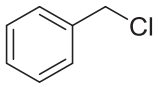
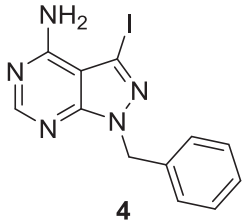
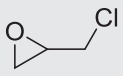
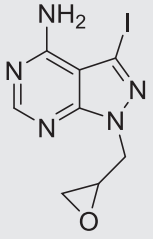
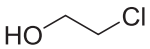
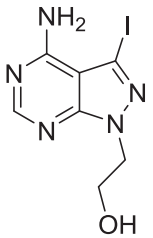
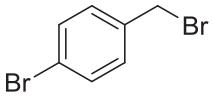
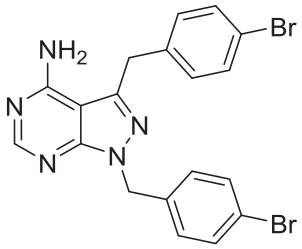
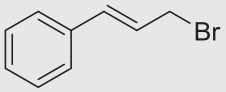
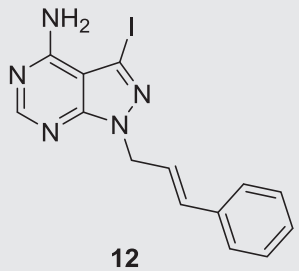
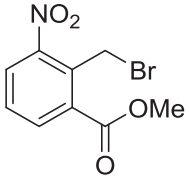
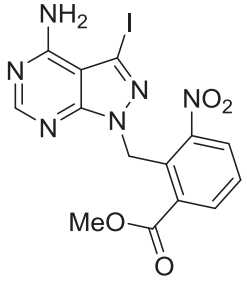
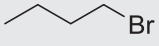
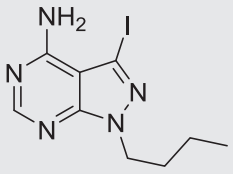
Entry	Reagent	*N*-Alkylated product	Yield (%)
1	 **3**	 **4**	44
2	 **5**	 **6**	No reaction
3	 **7**	 **8**	No reaction

TABLE 2 *N*-Alkylated pyrazolopyrimidines from alkyl bromide

Entry	Reagent	*N*-Alkylated product	Yield (%)
1	 **9**	 **10**	87
2	 **11**	 **12**	79
3	 **13**	 **14**	70
4	 **15**	 **16**	57

alkylation of pyrazolo[3,4-*d*]pyrimidine without any purification. Products **18**, **20**, **22**, **24**, **26**, **28**, **30**, and **32** were obtained with 67–79% yield rates, as seen in Table 3.

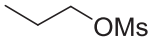
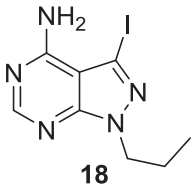
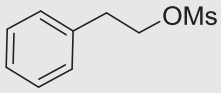
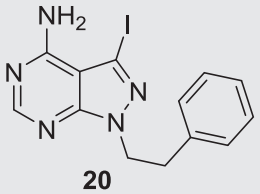
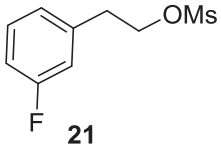
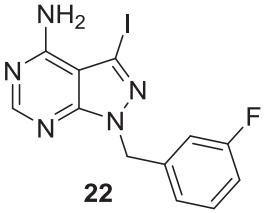
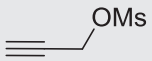
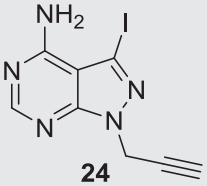
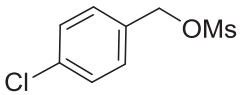
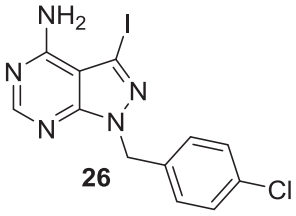
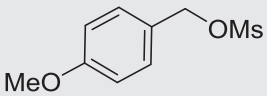
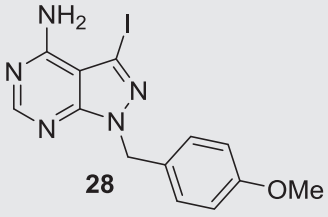
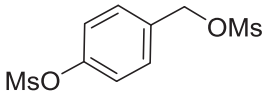
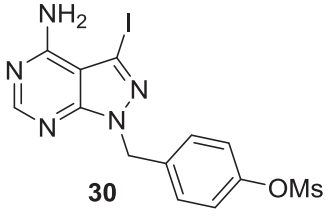
2.2 | Pharmacology/biology

2.2.1 | Biological evaluation

The novel synthesized 13 *N*-alkylated pyrazolo[3,4-*d*]pyrimidine derivatives (**4**, **10**, **12**, **14**, **16**, **18**, **20**, **22**, **24**, **26**, **28**, **30**, and **32**) were tested against AChE (associated with AD) and cytosolic isoforms hCA I and II (associated with glaucoma and epilepsy) by using the Ellman's and esterase assay methods. The standard inhibitors, tacrine (TAC) and acetazolamide (AZA) were employed as a reference for AChE and hCA isoforms, respectively. According to Tables 4 and 5, it is shown that all *N*-alkylated pyrazolo[3,4-*d*]pyrimidine derivatives are potent inhibitors against AChE, hCA I, and II. The inhibition data (IC_{50} values and K_i values) are listed in the tables.

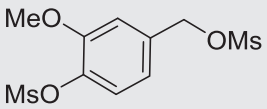
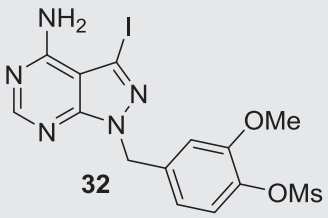
1. All the novel synthesized *N*-alkylated pyrazolo[3,4-*d*]pyrimidine derivatives moderately inhibited cytosolic isoform hCA I, with

TABLE 3 *N*-Alkylated pyrazolopyrimidines from alkyl mesylate

Entry	Reagent	*N*-Alkylated product	Yield (%)
1	 **17**	 **18**	75
2	 **19**	 **20**	66
3	 **21**	 **22**	65
4	 **23**	 **24**	64
5	 **25**	 **26**	67
6	 **27**	 **28**	74
7	 **29**	 **30**	79

(Continues)

TABLE 3 (Continued)

Entry	Reagent	*N*-Alkylated product	Yield (%)
8	 **31**	 **32**	75

IC_{50} values ranging from 16.12 to 69.30 nM and K_i values ranging from 17.68 ± 1.92 to 66.27 ± 5.43 nM. Moreover, all compounds displayed the best inhibition values, compared with AZA, which is a standard inhibitor, and they were found to be more effective inhibitors in the range between 7.16 and 26.90 as compared with AZA (K_i: 475.55 ± 10.62 nM). Compound **10** displayed the best inhibition values (K_i: 17.68 ± 1.92 nM) (Figure 2a). Compound **32** displayed low inhibition values according to results. The inhibitor properties of *N*-alkylated pyrazolo[3,4-*d*]pyrimidines against hCA I were found to be decreased in the following order: **10** > (K_i: 17.68 ± 1.92 nM) **18** > (K_i: 19.51 ± 3.33 nM) **14** > (K_i: 19.75 ± 3.95 nM) **22** > (K_i: 20.46 ± 4.98 nM) **20** > (K_i: 23.10 ± 2.37 nM) **12** > (K_i: 30.76 ± 0.72 nM) **26** > (K_i: 31.60 ± 3.46 nM) **4** > (K_i: 33.75 ± 4.66 nM) **28** > (K_i: 35.22 ± 6.22 nM) **16** > (K_i: 44.25 ± 5.32 nM) **24** > (K_i: 51.35 ± 7.32 nM) **30** > (K_i: 60.44 ± 3.89 nM) **32** > (K_i: 66.27 ± 5.43 nM) (Tables 4 and 5 and Figure 3a; the corresponding plots are given in the Supporting Information). According to results, the addition of an ethyl group to compound **4** caused an increase in the inhibition effect against hCA I (compound **20**, K_i: 23.10 ± 2.37 nM). The replacement of the 4-chlorobenzyl (**26**, K_i: 31.60 ± 3.46 nM) moieties with methoxy (**28**, K_i: 35.22 ± 11.77 nM) and methanesulfonate (**30**, K_i: 60.44 ± 3.89 nM) groups caused a decrease in the inhibition values. The reduction in the length of the alkyl chain at N2 from butyl (**16**) to propyl (**18**) led to a decrease in the inhibitory activity. Finally, the addition of a methoxy group to compound **30** led to a more active analog (**32**, K_i: 66.27 ± 5.43 nM).

2. *N*-Alkylated pyrazolo[3,4-*d*]pyrimidine derivatives effectively inhibited cytosolic isoform hCA II, with IC_{50} values ranging from 12.83 to 36.47 nM and K_i values ranging from 8.41 ± 2.03 to 28.60 ± 7.32 nM. These 13 analogs displayed a better inhibition effect than the standard inhibitor AZA (K_i: 97.73 ± 1.62 nM). Compound **14** (K_i: 8.41 ± 2.03 nM) showed the best inhibitory profile (Figure 2b),

TABLE 4 IC_{50} and K_i values of hCA isoforms (I–II) and AChE with *N*-alkylated pyrazolo[3,4-*d*]pyrimidine compounds

	IC_{50} (nM)						K_i (nM)		
Compounds	hCA I	r^2	hCA II	r^2	AChE	r^2	hCA I	hCA II	AChE
4	49.50	0.9847	28.88	0.9902	36.47	0.9740	33.75 ± 4.66	26.15 ± 3.15	33.04 ± 5,12
10	25.67	0.9845	18.73	0.9851	23.10	0.9869	17.68 ± 1.92	18.24 ± 2.77	22.30 ± 3.94
12	33.00	0.9844	30.13	0.9776	33.00	0.9811	30.76 ± 0.72	26.65 ± 4.64	31.81 ± 0.79
14	19.80	0.9946	16.12	0.9911	40.76	0.9858	19.75 ± 3.95	8.41 ± 2.03	37.28 ± 3.78
16	51.44	0.9822	23.90	0.9790	34.65	0.9934	44.25 ± 5.32	9.90 ± 2.21	32.56 ± 4.41
18	34.65	0.9834	31.50	0.9885	25.67	0.9841	19.51 ± 3.33	17.18 ± 0.66	18.62 ± 2.70
20	40.76	0.9876	33.00	0.9812	28.88	0.9781	23.10 ± 2.37	15.61 ± 0.87	24.97 ± 5.87
22	38.50	0.9852	34.65	0.9738	31.50	0.9967	20.46 ± 4.98	16.44 ± 3,72	27.75 ± 5.20
24	53.31	0.9945	36.47	0.9822	49.50	0.9909	51.35 ± 7.32	15.48 ± 0.40	49.03 ± 4.21
26	43.41	0.9844	31.50	0.9945	69.30	0.9854	31.60 ± 3.46	28.60 ± 7.32	59.12 ± 9.74
28	36.47	0.9849	27.72	0.9893	73.65	0.9796	35.22 ± 6.22	24.45 ± 4.73	63.03 ± 10.68
30	66.00	0.9960	23.10	0.9808	63.00	0.9911	60.44 ± 3.89	10.61 ± 0.87	55.27 ± 11.29
32	69.30	0.9849	12.83	0.9806	20.38	0.9978	66.27 ± 5.43	11.55 ± 1.21	15.41 ± 1.39
AZA[a]	223.90	0.9988	96.67	0.9999	–	–	475.55 ± 10.62	97.73 ± 1.62	–
TAC[b]	–	–	–	–	430.10	0.9998	–	–	159.64 ± 0.87

Abbreviations: AChE, acetylcholinesterase; AZA, acetazolamide; hCA, human carbonic anhydrase; TAC, tacrine.

[a]AZA was used as a positive control for carbonic anhydrase enzyme.[27]

[b]TAC was used as a positive control for acetylcholinesterase enzyme.[27]

whereas compound **26** (K_i: 28.60 ± 7.32 nM) showed the weakest inhibition effect against hCA II. The inhibitory properties of *N*-alkylated pyrazolo[3,4-*d*]pyrimidine derivatives against hCA II were found to be decreased in the following order: **14** > (K_i: 8.41 ± 2.03 nM) **16** > (K_i: 9.90 ± 2.21 nM) **30** > (K_i: 10.61 ± 0.87 nM) **32** > (K_i: 11.55 ± 1.21 nM) **24** > (K_i: 15.48 ± 0.40 nM) **20** > (K_i: 15.61 ± 0.87 nM) **22** > (K_i: 16.44 ± 3.72 nM) **18** > (K_i: 17.18 ± 0.66 nM) **10** > (K_i: 18.24 ± 2.77 nM) **28** > (K_i: 24.45 ± 4.73 nM) **4** > (K_i: 26.15 ± 3.15 nM) **12** > (K_i: 26.65 ± 4.64 nM) **26** > (K_i: 28.60 ± 7.32 nM) (Tables 4 and 5 and Figure 3b). As a general trend, comparing compound **18** with **20**, it is observed that the addition of a phenyl group increased the inhibition properties (**20**, K_i: 15.61 ± 0.87 nM). A similar situation is observed in hCA I inhibition. The replacement of the 4-methanesulfonate (**30**, K_i: 10.61 ± 0.87 nM) moiety with chlorobenzyl (**26**, K_i: 28.60 ± 7.32 nM), and methoxybenzyl (**28**, K_i: 24.45 ± 4.73 nM) groups caused a decrease in the inhibition values.

3. All the synthesized novel series of *N*-alkylated pyrazolo[3,4-*d*]pyrimidines showed potent inhibitory activity against the AChE enzyme with IC_{50} values ranging from 20.38 to 73.65 nM and K_i values ranging from 15.41 ± 1.39 to 63.03 ± 10.68 nM. Herein, compound **32** displayed the highest inhibition values, with K_i constant of 15.41 ± 1.39 nM (Figure 2c). This value is 10.36 times stronger than the standard inhibitor (TAC, K_i: 159.64 ± 0.87 nM). When analyzing the K_i values of the substances and examining the inhibitory effects, *N*-alkylated pyrazolo[3,4-*d*]pyrimidines showed a better inhibition effect on the AChE enzyme. The inhibitor properties of *N*-alkylated pyrazolo[3,4-*d*]pyrimidines against AChE were found to be decreased in the following order: **32** > (K_i: 15.41 ± 1.39 nM) **18** > (K_i: 18.62 ± 2.70 nM) **10** > (K_i: 22.30 ± 3.94 nM) **20** > (K_i: 24.97 ± 5.87 nM) **22** > (K_i: 27.75 ± 5.20 nM) **12** > (K_i: 31.81 ± 0.79 nM) **16** > (K_i: 32.56 ± 4.41 nM) **4** > (K_i: 33.04 ± 5.12 nM) **14** > (K_i: 37.28 ± 3.78 nM) **24** > (K_i: 49.03 ± 4.21 nM) **30** > (K_i: 55.27 ± 11.29 nM) **26** > (K_i: 59.12 ± 9.74 nM) **28** > (K_i: 63.03 ± 10.68 nM) (Tables 4 and 5 and Figure 3c). As a general trend, comparing compound **30** with compound **32**, it is observed that the addition of a methoxy group caused a 3.59-fold decrease in the inhibitory properties (**30**, K_i: 55.27 ± 11.29 nM). Compound **26** exhibited a more effective inhibition profile than compound **28**. According to this result, it can be said that the 4-chlorobenzyl group is more effective than the 4-methoxybenzyl in inhibiting the AChE activity. A similar result was observed with hCA I inhibition. The increase in the length of the group at N2 position from benzyl (**4**) to phenethyl (**20**) improved the inhibition against hCA II (compound **20**, K_i: 15.61 ± 0.87 nM).

TABLE 5 Selectivity index values for K_i constants of the *N*-alkylated pyrazolo[3,4-*d*]pyrimidine compounds

Compounds	K_i (AZA/hCA I)	K_i (AZA/hCA II)	K_i (TAC/AChE)
4	14.09	3.74	4.83
10	26.90	5.36	7.16
12	15.46	3.67	5.02
14	24.09	11.62	4.28
16	10.75	9.87	4.90
18	24.37	5.69	8.57
20	20.59	6.26	6.39
22	23.24	5.94	5.75
24	9.26	6.31	3.26
26	15.04	3.42	2.70
28	13.50	4.00	2.53
30	7.87	9.21	2.89
32	7.16	8.46	10.36

Abbreviations: AChE, acetylcholinesterase; AZA, acetazolamide; hCA, human carbonic anhydrase; TAC, tacrine.

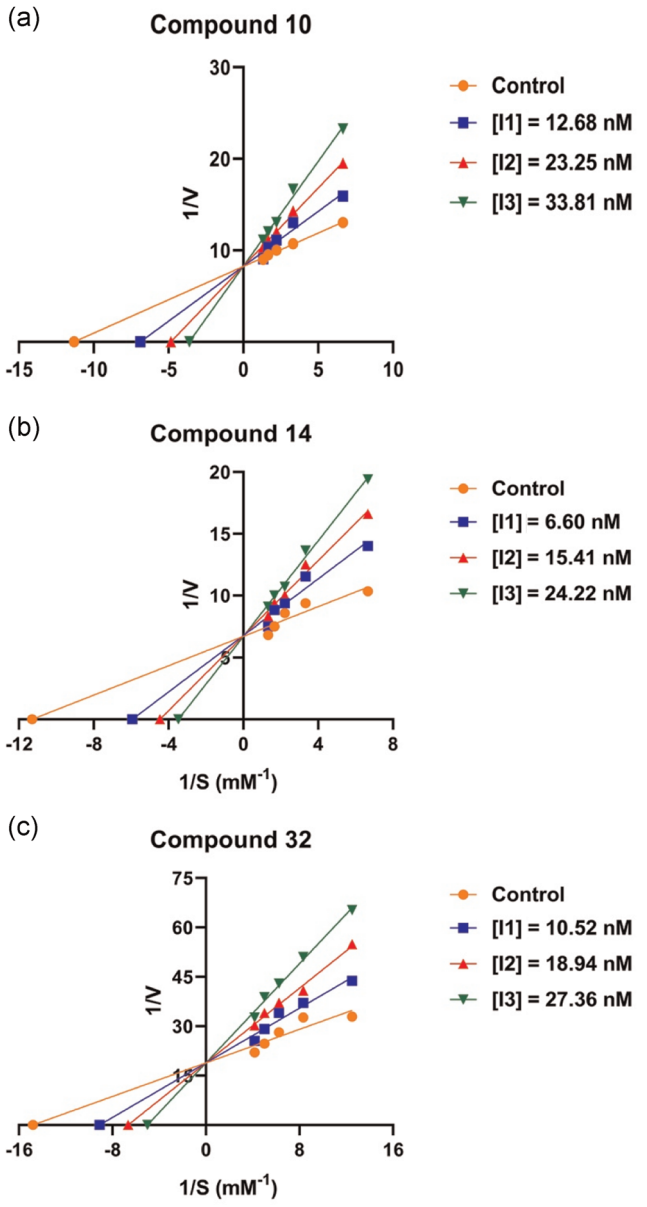


FIGURE 2 Lineweaver–Burk graphs for the best inhibitors. (a) Human carbonic anhydrase (hCA) I, (b) hCA II, (c) acetylcholinesterase

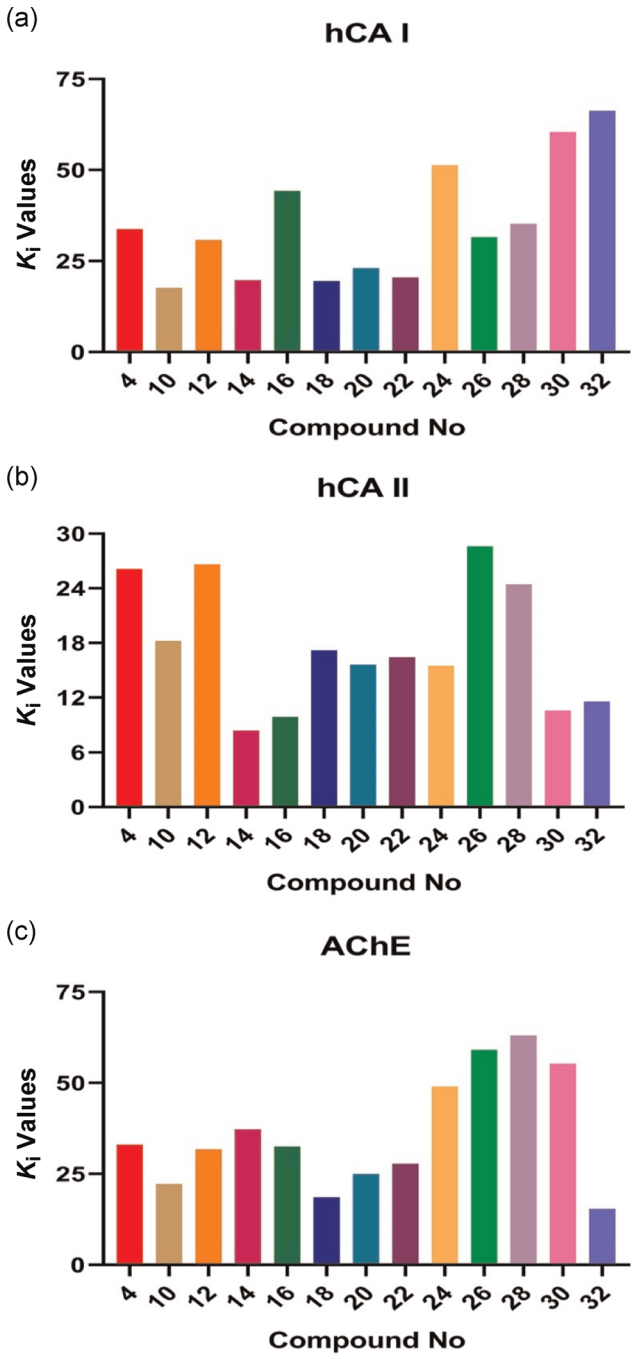


FIGURE 3 K_i values of the compounds for (a) human carbonic anhydrase (hCA) I, (b) hCA II, (c) acetylcholinesterase (AChE)

Similarly, the addition of a fluorine atom to compound **4** caused an increase in the inhibition effect against AChE (compound **22**, K_i: 27.75 ± 5.20 nM).

3 | CONCLUSION

This study reports an efficient protocol for the *N*-alkylation of compound 3-iodo-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (**2**). In total, 13 novel N-substituted pyrazolo[3,4-*d*]pyrimidine derivatives have been prepared and structurally characterized by ^{1}H NMR, ^{13}C NMR, and HRMS. These compounds were monitored in vitro for their inhibitory potential against AChE and hCA I and II isoenzymes. The biological studies, which were determined as significant targets in AD treatment, primarily, were performed using Ellman and Verpoorte methods. In vitro studies revealed that the synthesized compounds based on our design notably inhibited hCA I, hCA II, and AChE, even more than reference drugs, namely, AZA and TAC. Compound **10** displayed the best inhibition against hCA I and compound **14** displayed the best inhibition against hCA II. Moreover, compound **32** was defined as the most significant and selective AChE inhibitor in this series. Finally, all derivatives in this series can be considered outstanding multitarget inhibitors for further AD treatment investigations.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General information

All reactions were performed under a nitrogen atmosphere. All commercial reagents and chromatography solvents were used as obtained unless otherwise stated. Analytical thin-layer chromatography (TLC) was performed on Merck silica gel 60 F254. Visualization of TLCs was accomplished by UV light (254 nm) and staining with an ethanolic PMA (phosphomolybdic acid) solution. Elemental analysis was performed on a Leco CHNS-932. HRMS was performed using Agilent 7800 ICP-MS. Infrared (IR) spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer. Melting points were measured with Gallenkamp melting point devices. NMR spectra were recorded using a Bruker 400 MHz NMR instrument (^{1}H NMR at 400 MHz and ^{13}C NMR at 100 MHz) or a Varian 400 MHz instrument (^{1}H NMR at 400 MHz and ^{13}C NMR at 100 MHz) in a $CDCl_3$ solution using tetramethylsilane as an internal standard. ^{1}H NMR data are reported as follows: chemical shift (δ, ppm), multiplicity (s = singlet, d = dublet, t = triplet, q = quartet, dd = doublet of doublets, dt = doublet of triplets, m = multiplet or otherwise stated), integration, coupling constant (J, Hz). ^{13}C NMR data are given in terms of chemical shift (δ, ppm) (please see the Supporting Information for the original spectra).

The InChI codes of the investigated compounds, together with some biological activity data, are provided as Supporting Information.

4.1.2 | General procedure for the preparation of *N*-alkylated pyrazolo[3,4-*d*]pyrimidines 4, 10, 12, 14, and 16

Anhydrous K_2CO_3 (2 eq) was added to a mixture of 3-iodo-1*H*-pyrazolo [3,4-*d*]pyrimidin-4-amine (1 eq) and alkyl halide (**3**, **9**, **11**, **13**, **15**) (2 eq) in dry DMF (50 ml). The reaction mixture was stirred overnight at 70°C under a nitrogen atmosphere. After cooling, it was poured into water (80 ml). It was extracted with EtOAc (3 × 60 ml). The combined organic

layers were dried over Na_2SO_4 and concentrated under reduced pressure to give the crude product. The crude was precipitated (DCM/hexanes, 1:20, 40 ml) and filtered to give the desired product.

*1-Benzyl-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**4**)*

Yellow solid. Yield: 44%, m.p.: 214–216°C. IR (KBr, cm^{-1}): 3589 and 540. ^1H NMR (400 MHz, $CDCl_3$) δ 8.39 (s, 1H), 7.39–7.29 (m, 5H), and 5.57 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.6, 156.6, 156.5, 154.1, 136.3, 128.9, 104.3, 86.8, and 51.5. Q-TOF LC/MS: 352.0044 ([M +H]$^+$, $C_{12}H_{11}IN_5^+$; calc: 352.0059).

*1,3-Bis(4-bromobenzyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**10**)*

White solid. Yield: 57%, m.p.: 190–192°C. IR (KBr, cm^{-1}): 3412 and 655. ^1H NMR (400 MHz, dimethyl sulfoxide [DMSO]) δ 8.27 (s, 1H), 7.51–7.48 (m, 4H), 7.30 (d, J = 8.2 Hz, 2H), 7.17 (d, J = 8.2 Hz, 2H), 5.46 (s, 2H), and 4.77 (d, J = 5.9 Hz, 2H). ^{13}C NMR (100 MHz, DMSO) δ 156.9, 156.7, 153.9, 139.4, 136.8, 132.3, 131.9, 130.5, 129.98, 121.6, 104.0, 89.7, 50.1, and 43.6. Q-TOF LC/MS: 471. 9770, ([M +H]$^+$; $C_{19}H_{16}Br_2N_5^+$; calculated: 471.9772).

*(E)-3-Iodo-1-styryl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**12**)*

White solid. Yield: 70%, m.p.: 182–184°C. IR (KBr, cm^{-1}): 3450, 1651, and 610. ^1H NMR (400 MHz, DMSO) δ 8.21 (s, 1H), 7.41 (d, J = 7.2 Hz, 1H), 7.32–7.16 (m, 3H), 6.55 (d, J = 15.9 Hz, 1H), 6.40 (dt, J = 15.9, 6.1 Hz, 2H), and 5.04 (d, J = 6.1 Hz, 2H). ^{13}C NMR (100 MHz, DMSO) δ 158.4, 156.8, 154.0, 136.5, 133.4, 129.3, 128.6, 127.2, 124.9, 103.8, and 90.0. Elemental analysis for $C_{14}H_{12}IN_5$: Calculated: C, 44.58; H, 3.21; N, 18.57; found: C, 44.47; H, 3.49; N, 18.26.

*Methyl 2-[(4-amino-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-1-yl) methyl]-3-nitrobenzoate (**14**)*

Yellow solid. Yield: 70%, m.p.: 254–256°C. IR (KBr, cm^{-1}): 3445, 1727, 1555, 1286, and 641. ^1H NMR (400 MHz, DMSO) δ 8.18 (s, 1H), 8.11 (d, J = 8.0 Hz, 1H), 8.01 (d, J = 8.0 Hz, 1H), 7.72 (t, J = 8.0 Hz, 1H), 5.89 (s, 2H), and 3.78 (s, 3H). ^{13}C NMR (100 MHz, DMSO) δ 167.0, 158.3, 156.8, 154.2, 151.8, 135.1, 130.72, 128.6, 105.8, 103.5, 91.2, 87.9, 53.6, and 43.1. Q-TOF LC/MS: 454.9947, ([M+H]$^+$; $C_{14}H_{12}IN_6O_4^+$; calculated: 454.9964).

*1-Butyl-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**16**)*

White solid. Yield: 87%, m.p.: 168–170°C. IR (KBr, cm^{-1}): 3444, 1488, and 610. ^1H NMR (400 MHz, DMSO) δ 8.17 (s, 1H), 4.24 (t, J = 7.4 Hz, 2H), 1.82–1.60 (m, 2H), 1.30–1.07 (m, 2H), and 0.84 (t, J = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, DMSO) δ 158.3, 156.6, 154.0, 103.6, 89.2, 46.9, 31.8, 19.9, and 14.1. Elemental analysis for $C_9H_{12}IN_5$: Calculated: C, 34.32; H, 3.90; N, 22.03,; found: C, 34.09; H, 3.81; N, 22.08.

4.1.3 | General procedure for the preparation of *N*-alkylated pyrazolo[3,4-*d*]pyrimidines 18, 20, 22, 24, 26, 28, 30, 32

NEt_3 (2 eq) and MsCl (2 eq) were added dropwise to a solution of R-OH (1 eq) in dry DCM (60 ml) under a nitrogen atmosphere. The reaction mixture was stirred for 22 h at room temperature, and then it was quenched with sat. NH_4Cl (40 ml) solution and extracted with DCM (2 × 50 ml). The combined organic layers were dried over Na_2SO_4 and filtered. The solvent was evaporated to dryness under reduced pressure to give alkyl methanesulfonate (**17**, **19**, **21**, **23**, **25**, **29**, **31**) (82–99%). The yellow liquid was used in the next step without purification.

Anhydrous K_2CO_3 (2 eq) was added to a mixture of 3-iodo-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (1 eq) and alkyl methanesulfonate (**17**, **19**, **21**, **23**, **25**, **29**, **31**) (2 eq) in dry DMF (50 ml). The reaction mixture was stirred overnight at 70°C under a nitrogen atmosphere. After cooling, it was poured into water (80 ml). It was extracted with EtOAc (3 × 60 ml). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure to give the crude product. The crude was precipitated (DCM/hexanes, 1:20, 40 ml) and filtered to give the desired product.

*3-Iodo-1-propyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**18**)*

White solid. Yield: 75%, m.p.: 207–209°C. IR (KBr, cm^{-1}): 3428, 1482, and 541. ^1H NMR (400 MHz, DMSO) δ 8.17 (s, 1H), 4.20 (t, J = 7.2 Hz, 2H), 1.77 (q, 7.2 Hz, 2H), and 0.78 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO) δ 158.4, 156.7, 154.1, 103.7, 89.3, 48.8, 23.2, and 11.6. Elemental analysis for $C_8H_{10}IN_5$: Calculated: C, 31.70; H, 3.33; N, 23.11; found: C, 31.03; H, 3.16; N, 23.21.

*3-Iodo-1-phenethyl-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**20**)*

Yellow solid. Yield: 66%, m.p.: 190–192°C. IR (KBr, cm^{-1}): 3438, 3368, 1492, and 539. ^1H NMR (400 MHz, CDCl3) δ 8.29 (s, 1H), 7.32–7.15 (m, 5H), 6.29 (bs, 2H), 4.60 (t, 2H), and 3.21 (t, 2H). ^{13}C NMR (100 MHz, CDCl3) δ 157.5, 156.0, 153.8, 137.6, 128.8, 126.7, 103.9, 86.0, 48.8, and 36.0. Elemental analysis for $C_{13}H_{12}IN_5$: Calculated: C, 42.76; H, 3.31; N, 19.18; found: C, 43.13; H, 3.36; N, 19.28.

*1-(3-Fluorobenzyl)-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**22**)*

White solid. Yield: 65%, m.p.: 208–210°C. IR (KBr, cm^{-1}): 3454, 1101, and 602. ^1H NMR (400 MHz, DMSO) δ 8.22 (s, 1H), 7.34 (d, J = 8.3 Hz, 1H), 7.24–7.05 (m, 3H), and 5.51 (s, 2H). ^{13}C NMR (100 MHz, DMSO) δ 161.8, 159.3, 158.4, 157.0, 154.3, 125.3, 124.3, 124.1, 116.0, 103.7, 90.6, and 44.5. Elemental analysis for $C_{12}H_9FIN_5$: Calculated: C, 39.05; H, 2.46; N, 18.97; found: C, 39.11; H, 2.61; N, 18.74.

*3-Iodo-1-(prop-2-yn-1-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**24**)*

Yellow solid. Yield: 64%, m.p.: 220–222°C. IR (KBr, cm^{-1}): 3439, 3042, and 597. ^1H NMR (400 MHz, DMSO) δ 8.21 (s, 1H), 5.10 (d, J = 2.3 Hz, 2H), and 3.39 (t, J = 2.3 Hz, 1H). ^{13}C NMR (100 MHz, DMSO) δ 158.4, 157.1, 153.8, 103.8, 91.4, 78.9, 76.4, and 36.7. Q-TOF LC/MS: 299.9746 $C_8H_7IN_5^+$; ([M+H]$^+$ calculated: 299.9746.)

*1-(4-Chlorobenzyl)-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-4-amine (**26**)*

White solid. Yield: 67%, m.p.: 249–251°C. IR (KBr, cm^{-1}): 3445, and 585. ^1H NMR (400 MHz, DMSO) δ 8.22 (s, 1H), 7.37 (d, J = 8.4 Hz, 2H), 7.23 (d, J = 8.4 Hz, 2H), and 5.46 (s, 2H). ^{13}C NMR (100 MHz,

DMSO) δ 158.4, 157.1, 154.2, 136.6, 133.0, 130.3, 129.4, 103.8, 90.6, and 49.9. Elemental analysis for $C_{12}H_9ClIN_5$: Calculated: C, 37.38; H, 2.35; N, 18.16; found: C, 37.08; H, 2.69; N, 18.21.

*3-Iodo-1-(4-methoxybenzyl)−1H-pyrazolo[3,4-d]pyrimidin-4-amine (**28**)*

White solid. Yield: 74%, m.p.: 247–249°C. IR (KBr, cm^{-1}): 3448, 1246, and 538. ^1H NMR (400 MHz, $CDCl_3$) δ 8.39 (s, 1H), 7.36 (d, J = 8.8 Hz, 2H), 6.86 (d, J = 8.8 Hz, 2H), 5.50 (s, 2H), and 3.79 (s, 3H). ^{13}C NMR (100 MHz, DMSO) δ 159.5, 158.4, 157.0, 156.8, 130.0, 129.8, 114.7, 103.8, 90.1, 55.7, and 50.2. Quadrupole time-of-flight liquid chromatography–mass spectrometry (Q-TOF LC/MS): 382.0138 $C_{13}H_{13}IN_5O^+$ ([M+H]$^+$ calculated: 382.0164).

*4-[(4-Amino-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl]phenyl methanesulfonate (**30**)*

White solid. Yield: 79%, m.p.: 209–211°C. IR (KBr, cm^{-1}): 3440, 1366, and 527. ^1H NMR (400 MHz, DMSO) δ 8.22 (s, 1H), 7.52–7.02 (t, J = 7.3 Hz, 4H), 5.50 (s, 2H). ^{13}C NMR (100 MHz, DMSO) δ 158.4, 157.0, 154.2, 149.1, 136.8, 130.1, 123.3, 103.8, 90.6, 49.9, and 38.1. Elemental analysis for $C_{13}H_{12}IN_5O_3S$: Calculated: 35.07; H, 2.72; N, 15.73; found: C, 35.09; H, 2.86; N, 15.58.

*4-[(4-Amino-3-iodo-1H-pyrazolo[3,4-d]pyrimidin-1-yl)methyl]-2-methoxyphenyl methanesulfonate (**32**)*

White solid. Yield: 75%, m.p.: 193–195°C. IR (KBr, cm^{-1}): 3445, 1345, 1261, and 541. ^1H NMR (400 MHz, DMSO) δ 8.23 (s, 1H), 7.30–7.14 (m, 2H), 6.71 (d, J = 8.3 Hz, 1H), 5.74 (s, 2H), 3.79 (s, 3H), and 3.31 (s, 3H). ^{13}C NMR (100 MHz, DMSO) δ 158.4, 157.1, 154.3, 152.0, 137.9, 124.8, 120.3, 113.6, 103.8, 90.5, 56.7, 56,5 50.3, and 49.0. Elemental analysis for $C_{14}H_{14}IN_5O_4S$: Calculated, %: C, 35.38; H, 2.97; N, 14.74; found: C, 34.98; H, 3.30; N, 14.38.

4.2 | Biological assays

4.2.1 | Biological studies

CA isoforms (I and II) were purified from human erythrocytes by Sepharose-4B-L-tyrosine–sulfanilamide affinity chromatography, as in a previous study.[44] The protein concentration of the eluates was determined by a straightforward analytical procedure at 595 nm according to the Bradford method, spectrophotometrically.[45] The purity of the purified enzyme fractions of both isoenzymes was determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis,[46,47] which was realized on 8% slab gels,[48] as explained by Laemmli.[49] The esterase activity of human erythrocyte CA isoforms was determined according to the method defined by Verpoorte et al.[50] All rate measurements were conducted in triplicate. IC_{50} and K_i values were computed as in previous studies.[51–53] The inhibition type for each of the derivatives was found using Lineweaver–Burk curves.[54]

4.2.2 | AChE activity assay

Acetylcholinesterase from *Electrophorus electricus* (C2888, Type V-S) was used in this study. In vitro effects on the AChE activity of the target *N*-alkylated pyrazolo[3,4-*d*]pyrimidine derivatives (**4**, **10**, **12**, **14**, **16**, **18**, **20**, **22**, **24**, **26**, **28**, **30**, **32**) were evaluated by the method of Ellman et al.[55] TAC was used as the reference drug. All the measurements were repeated three times.

ORCID

Busra O. Aydin https://orcid.org/0000-0002-0045-5254
Derya Anil https://orcid.org/0000-0003-2584-275X
Yeliz Demir https://orcid.org/0000-0003-3216-1098

REFERENCES

[1] M. Hossain, A. K. Nanda, *J. Chem. Sci.* **2018**, *6*(5), 83.
[2] I. Matos, E. Perez-Mayoral, E. Soriano, A. Zukal, R. M. Martin-Aranda, A. J. Lopez-Peinado, I. Fonseca, J. Ceyka, *Chem. Eng.* **2010**, *161*, 377.
[3] R. Merugu, S. Garimella, D. Balla, K. Sambaru, *Int. J. PharmTech. Res.* **2015**, *8*(6), 88.
[4] R. Aggarwal, S. Kumar, *Beilstein J. Org. Chem.* **2018**, *14*, 203.
[5] S. B. Yewale, S. B. Ganorkar, K. G. Baheti, R. U. Shelke, *Bioorg. Med. Chem. Lett.* **2012**, *22*, 6616.
[6] M. M. El-Enany, M. M. Kamel, O. M. Khalil, H. B. El-Nassan, *Eur. J. Med. Chem.* **2010**, *45*, 5286.
[7] F. Manetti, A. Santucci, G. A. Locatelli, G. Maga, A. Spreafico, T. Serchi, M. Orlandini, G. Bernardini, N. P. Caradonna, A. Spallarossa, C. Brullo, S. Schenone, O. Bruno, A. Ranise, F. Bondavalli, O. Hoffmann, M. Bologna, A. Angelucci, M. Botta, *J. Med. Chem.* **2007**, *50*, 5579.
[8] Y. Huang, L. Wang, C. Chang, Y. Kuo, K. Kaneko, H. Takayama, M. Kimura, S. Juang, F. F. Wong, *Tetrahedron* **2012**, *68*, 9658.
[9] M. Maher, A. E. Kassab, A. F. Z., Z. Mahmoud, *J. Enzyme Inhib. Med. Chem.* **2019**, *34*, 532.
[10] K. Verschueren, M. Cobbaut, J. Demaerel, L. Saadah, A. R. D. Voet, J. V. Lint, W. M. D. Borggraeve, *MedChemComm* **2017**, *8*, 640.
[11] J. C. Verheijen, D. J. Richard, K. Curran, J. Kaplan, M. Lefever, P. Nowak, D. J. Malwitz, N. Brooijmans, L. Toral-Barza, W. Zhang, J. Lucas, I. Hollander, S. Ayral-Kaloustian, T. S. Mansour, K. Yu, A. Zask, *J. Med. Chem.* **2009**, *52*, 8010.
[12] D. J. Baillache, A. Unciti-Broceta, *RSC Med. Chem.* **2020**, *11*, 1112.
[13] S. Gaonkar, M. Azharuddin Savanur, A. A. Nadaf, M. S. Najare, S. Mantur, M. Garbhagudi, S. I. Mulla, I. A. M. Khazi, *Arch. Pharm.* **2020**, *353*(4), 1900296.
[14] B. Sever, C. Türkeş, M. D. Altıntop, Y. Demir, Ş. Beydemir, *Int. J. Biol. Macromol.* **2020**, *163*, 1970.
[15] E. Hoff, D. Zou, S. Schiza, Y. Demir, L. Grote, I. Bouloukaki, J. Hedner, *J. Sleep Res.* **2020**, *29*(2), e12956.
[16] C. Caglayan, Y. Demir, S. Kucukler, P. Taslimi, F. M. Kandemir, İ. Gulçin, *J. Food Biochem.* **2019**, *43*(2), e12720.
[17] P. Taslimi, F. M. Kandemir, Y. Demir, M. İleritürk, Y. Temel, C. Caglayan, İ. Gulçin, *J. Biochem. Mol. Toxicol.* **2019**, *33*(6), e22313.
[18] F. Erdemir, D. B. Celepci, A. Aktaş, Y. Gök, R. Kaya, P. Taslimi, İ. Gulçin, *Bioorg. Chem.* **2019**, *91*, 103134.
[19] C. Çağlayan, P. Taslimi, Y. Demir, S. Küçükler, F. M. Kandemir, İ. Gulçin, *J. Biochem. Mol. Toxicol.* **2019**, *33*(10), e22381.
[20] C. Caglayan, P. Taslimi, C. Türk, F. M. Kandemir, Y. Demir, İ. Gulcin, *Comp. Biochem. Phys. C* **2019**, *226*, 108605.
[21] H. E. Aslan, Y. Demir, M. S. Özaslan, F. Türkan, Ş. Beydemir, Ö. İ. Küfrevioğlu, *Drug Chem. Toxicol.* **2019**, *42*(6), 634.

[22] C. Türkeş, M. Arslan, Y. Demir, L. Cocaj, A. R. Nixha, Ş. Beydemir, *Bioorg. Chem.* **2019**, *89*, 103004.
[23] S. Gündoğdu, C. Türkeş, M. Arslan, Y. Demir, Ş. Beydemir, *ChemistrySelect* **2019**, *4*(*45*), 13347.
[24] B. N. Sağlık, U. A. Çevik, D. Osmaniye, S. Levent, B. K. Çavuşoğlu, Y. Demir, Z. A. Kaplancıklı, *Bioorg. Chem.* **2019**, *91*, 103153.
[25] M. Işık, Y. Demir, M. Durgun, C. Türkeş, A. Necip, Ş. Beydemir, *Chem. Pap.* **2020**, *74*(*5*), 1395.
[26] M. Durgun, C. Türkeş, M. Işık, Y. Demir, A. Saklı, A. Kuru, Z. AlOthman, *J. Enzyme Inhib. Med. Chem.* **2020**, *35*(*1*), 950.
[27] Q. Istrefi, C. Türkeş, M. Arslan, Y. Demir, A. R. Nixha, Ş. Beydemir, Ö. İ. Küfrevioğlu, *Arch. Pharm.* **2020**, *353*(*6*), 1900383.
[28] C. Yamalı, H. I. Gül, Y. Demir, C. Kazaz, İ. Gulcin, *Turk. J. Chem.* **2020**, *44*, 1058.
[29] C. Yamalı, H. I. Gül, T. Cakır, Y. Demir, İ. Gulcin, *Lett. Drug Des. Discov.* **2020**, *17*(*10*), 1283.
[30] M. Kalayci, C. Türkeş, M. Arslan, Y. Demir, Ş. Beydemir, *Arch. Pharm.* **2020**, e2000282. https://doi.org/10.1002/ardp.202000282
[31] N. Lolak, S. Akocak, C. Türkeş, P. Taslimi, M. Işık, Ş. Beydemir, M. Durgun, *Bioorg. Chem.* **2020**, *100*, 103897.
[32] H. Zhi, C. Zhang, Z. Cheng, Z. Jin, E. Huang, S. Li, H. Lin, D. C. Wan, C. Hu, *Med. Chem.* **2013**, *9*(*5*), 703.
[33] H. Kuday, F. Sonmez, C. Bilen, E. Yavuz, N. Gençer, M. Kucukislamoglu, *BioMed. Res. Int.* **2014**, *2014*, 594879.
[34] S. Harma, V. J. Shukla, *Int. J. Pharm. Pharm. Sci.* **2019**, *11*(*3*), 66.
[35] C. Wang, H. Liu, Z. Song, Y. Ji, L. Xing, X. Peng, X. Wang, J. Ai, M. G. A. Zhang, *Bioorg. Med. Chem. Lett.* **2017**, *27*, 2544.
[36] N. Zheng, Q. Hao, K. Lin, J. Pana, Y. Li, W. Zhou, *Bioorg. Med. Chem. Lett.* **2019**, *29*, 225.
[37] N. Atatreh, A. M. Youssef, M. A. Ghattas, M. A. Sorkhy, S. Alrawashdeh, K. B. Al-Harbi, I. M. El-Ashmawy, T. I. Almundarij, A. A. Abdelghani, A. S. Abd-El-Aziz, *Bioorg. Chem.* **2019**, *86*, 393.
[38] A. L. Fallacara, R. Passannanti, M. Mori, G. Iovenitti, F. Musumeci, C. Greco, E. Crespan, M. Kissova, G. Maga, C. Tarantelli, F. Spriano, E. Gaudio, F. Bertoni, M. Botta, S. Schenone, *Eur. J. Med. Chem.* **2019**, *181*, 111545.
[39] N. S. M. Ismail, E. M. H. Ali, D. A. Ibrahim, R. A. T. Serya, D. A. A. E. Ella, *Fut. J. Pharm. Sci.* **2016**, *2*, 20.
[40] B. C. Bookser, M. I. Weinhouse, A. C. Burns, A. N. Valiere, L. J. Valdez, P. Stanczak, J. Na, A. L. Rheingold, C. E. Moore, B. Dyck, *J. Org. Chem.* **2018**, *83*(*12*), 6334.
[41] N. Liu, S. Hoogendoorn, B. Kar, A. Kaptein, T. Barf, C. Driessen, D. V. Filippov, G. A. Marel, M. Stelt, H. S. Overkleeft, *Org. Biomol. Chem.* **2015**, *13*, 5147.
[42] N. Todorovic, E. Awuah, T. Shakya, G. D. Wright, A. Capretta, *Tetrahedron Lett.* **2011**, *52*, 5761.
[43] A. Assadieskandar, C. Yu, P. Maisonneuve, I. Kurinov, F. Sicheri, C. Zhang, *ACS Med. Chem. Lett.* **2019**, *10*(*7*), 1074.
[44] C. Türkeş, Y. Demir, Ş. Beydemir, *J. Biomol. Struct. Dyn.* **2020**, 1. https://doi.org/10.1080/07391102.2020.1806927
[45] M. M. Bradford, *Anal. Biochem.* **1976**, *72*, 248.
[46] M. Işık, Ş. Beydemir, Y. Demir, M. Durgun, C. Türkeş, A. Nasır, A. Necip, M. Akkuş, *Int. J. Biol. Macromol.* **2020**, *146*, 1111.
[47] Y. Demir, *J. Pharm. Pharmacol.* **2019**, *71*(*10*), 1576.
[48] Y. Demir, *Drug Dev. Res.* **2020**, *81*(*5*), 628.
[49] U. K. Laemmli, *Nature* **1970**, *227*, 680.
[50] J. A. Verpoorte, S. Mehta, J. T. Edsall, *J. Biol. Chem.* **1967**, *242*, 4221.
[51] Y. Demir, C. Türkeş, Ş. Beydemir, *Anti-Cancer Agents Med. Chem.* **2020**, *20*(*7*), 887.
[52] B. Sever, M. D. Altıntop, Y. Demir, G. Akalin Ciftci, S. ̧. Beydemir, A. Özdemir, *Bioorg. Chem.* **2020**, *102*, 104110.
[53] B. Sever, M. D. Altıntop, Y. Demir, M. Pekdogan, G. Akalin Ciftci, Ş. Beydemir, A. Özdemir, *J. Mol. Struct.* **2021**, *1224*, 129446.
[54] H. Lineweaver, D. Burk, *J. Am. Chem. Soc.* **1934**, *56*, 658. https://doi.org/10.1021/ja01318a036
[55] G. L. Ellman, K. D. Courtney, V. Andres, Jr, R. M. Featherstone, *Biochem. Pharmacol.* **1961**, *7*, 88.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Aydin BO, Anil D, Demir Y. Synthesis of *N*-alkylated pyrazolo[3,4-*d*]pyrimidine analogs and evaluation of acetylcholinesterase and carbonic anhydrase inhibition properties. *Arch Pharm.* 2021;354:e2000330. https://doi.org/10.1002/ardp.202000330