Received: 20 February 2020 | Revised: 4 March 2020 | Accepted: 13 March 2020

DOI: 10.1002/jhet.3985

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Synthesis, cytotoxic, and carbonic anhydrase inhibitory effects of new 2-(3-(4-methoxyphenyl)-5-(aryl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzo[*d*]thiazole derivatives

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Funding information
Ataturk University; TUBITAK, Grant/Award Number: 117S939

Abstract

2-(3-[4-Methoxyphenyl]-5-aryl-4,5-dihydro-1*H*-pyrazol-1-yl)benzo[*d*]thiazoles (**1b-7b**) were synthesized for the first time except **1b**, and spectral methods such as ^{1}H NMR, ^{13}C NMR and HRMS were utilized to illuminate the chemical structures of the synthesized compounds. Phenyl (**1b**), 2-methoxyphenyl (**2b**), 4-methoxyphenyl (**3b**), 4-methoxy-3-hydroxyphenyl (**4b**), 2,5-dimethoxyphenyl (**5b**), 3,4,5-trimethoxyphenyl (**6b**), or thiophene-2-yl (**7b**) was used as a aryl part. The inhibitory effects of the compounds were evaluated toward human carbonic anhydrase I and II enzymes (hCA I and hCA II). In vitro cytotoxic effects of the compounds against human oral squamous carcinomas and human normal oral cells were carried out via MTT. The compounds (**1b-7b**) had Ki values of 36.87 ± 11.62-66.24 ± 2.99 μM (hCA I) and 22.66 ± 1.41-89.95 ± 6.25 μM (hCA II). Compounds **1b** (Ki = 36.87 ± 11.62 μM) toward hCA I, **6b** (Ki = 22.66 ± 1.41 μM) toward hCA II had significant enzyme inhibitory potency. Compound **6b** had the highest tumor selectivity (TS = 29.3) and potency selectivity expression (PSE = 272.3) values. Therefore, compounds **1b** and **6b** with CAs inhibition effect and compound **6b** with the cytotoxicity may be forwarded to further studies as potent compounds.

1 | INTRODUCTION

Heterocyclic chemistry has great importance especially in the development of new drug candidates. Compounds including a pyrazoline scaffold are of great importance for heterocyclic chemistry and it is available in many bioactive molecules. Pyrazoline is a partially reduced form of pyrazole, which has two adjacent nitrogen atoms and an endocyclic double bond.[1,2]

Pyrazoline has quite different pharmacological activities such as antimicrobial,[3] anticancer,[4,5] anticonvulsant,[6] anti-depressant,[7] anti-oxidant,[8] anti-inflammatory,[9] anti-tubercular,[10] and carbonic anhydrase (CA, EC4.2.1.1) inhibiting activities.[11,12]

Cancer is a disease defined by rapid and uncontrolled proliferation of abnormal cells. Globally, cancer is the second leading disease that causes death after cardiovascular diseases. Resistance to drugs, lack of selectivity, and the emergence of side effects in chemotherapeutic agents are the biggest problems in the treatment of cancers. Hence, there is a need to enhance new effective and selective drugs in the treatment of cancer.[13,14]

Oral squamous cell carcinoma (OSCC) is the most common type of oral cancer.[15] Morbidity and functional impairments may occur during the treatment of the disease. Although there are improvements in surgical techniques, post-operative radiation, or chemotherapy, the diagnosis and prevention of this malignancy has not

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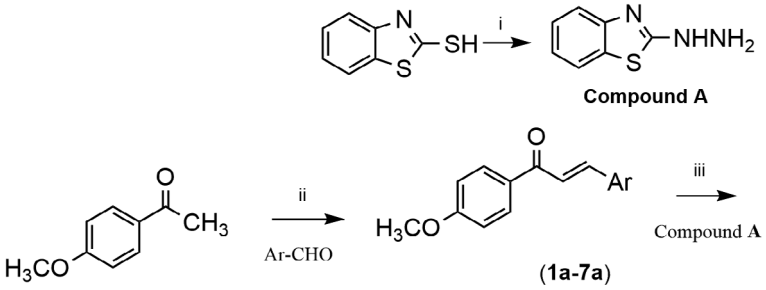
improved considerably.[16] Therefore, early diagnosis of OSCC is extremely important for the treatment of the disease.

CA is an enzyme responsible for the hydration of carbon dioxide (CO_2) and dehydration of HCO_3^-.[5,12,17–19] Carbonic anhydrases (CAs, E.C.4.2.1.1) are found in prokaryotes and eukaryotes. They take part in various physiological and pathological events, for instance, neurological disorders, bone resorption, pH regulation, gluconeogenesis, neurogenesis, glaucoma, lipogenesis, cancer, calcification, osteoporosis, tumorgenicity, and bicarbonate synthesis.

CA I and CA II are cytosolic CA isoenzymes. The most important function of these isoenzymes is their role in the respiratory event. Also, CA II plays a role in primary transport of sodium to the eye and helps regulate intraocular pressure. CA-IX and CA-XII isoenzymes are transmembrane proteins and are targeted for treatment in cancer. Their expression increases in response to hypoxia. They contribute to tumor development by triggering extracellular acidification. In addition, patients with Alzheimer's disease (AD) have been shown to have reduced CA levels in brain tissue significantly, which is evident that CA isozymes (hCA I, II, IV, and VII) play an important role in cognitive function.[18–21]

This study objected to synthesize new 2-(3-(4-methoxy phenyl)-5-aryl-4,5-dihydro-1*H*-pyrazol-1-yl) benzo[*d*]thiazole (**1b-7b**) to investigate their cytotoxic /anticancer activities against human oral squamous cell lines (Ca9-22, HSC-2, HSC-3, HSC-4) and normal oral cells (HGF, HPLF, HPC) and their CA (hCA I and hCA II) inhibition potency to ascertain their applicability as possible leading compounds.



SCHEME 1 The synthetic route for the synthesis of compounds **1b-7b**. i: Hydrazine hydrate (%80), ethanol, reflux, ii: NaOH (%10), ethanol, rt, iii:Ethanol, glacial acetic acid, reflux. Ar: Phenyl (**1b**), 2-methoxyphenyl (**2b**), 4-methoxyphenyl (**3b**), 4-methoxy-3-hydroxyphenyl (**4b**), 2,5-dimethoxyphenyl (**5b**), 3,4,5-trimethoxyphenyl (**6b**), thiophene-2-yl (**7b**)

2 | RESULTS AND DISCUSSION

The compounds (**1b-7b**) were synthesized for the first time except **1b**. The synthesis method of the target compounds was shown in Scheme 1. The cytotoxicity properties of the compounds were given in Table 1 and CAs inhibitory results are shown in Table 2.

Cytotoxic activities of the compounds were tested via MTT method.[20–26] The compounds had CC_{50} value of 3.1 - 400 μM toward OSCC cancer cell lines while the reference 5-fluorouracil (5-FU) had CC_{50} value of 7.8 and 261.0 μM. When the compounds cytotoxicities compared with 5-FU, against Ca9-22 cell line, **2b** (1.4 times) and **6b** (2.04 times) showed more cytotoxicity while **2b** (10.6 times), **4b** (13.5 times) and **6b** (10.03 times) were demonstrated favorable cytotoxicity against HSC-2 cells. Only compound **5b** (2.5 times) was cytotoxic against HSC-3 cells. Besides, compounds did not show any cytotoxicity against to HSC-4 cells.

Selectivity index (SI) value was calculated via equation of [Mean D] on normal cells / [CC_{50}] on OSCC and presented in Table 1. SI value (SI > 1) is considered as a key parameter to identify tumor-selective compounds.[20–26] According to Table 1, the SI values of the compounds toward a specific cell line, were as follows: [Ca9-22: **2b** (6.3), **4b** (13.8), **5b** (1.2), **6b** (28.5), **7b** (2.0)], [HSC-2: **2b** (3.9), **4b** (16.5), **6b** (11.8)], [HSC-3: **2b** (3.5), **4b** (19.5), **5b** (61.3), **6b** (18.6), **7b** (3.0)], [HSC-4: **2b** (2.6), **4b** (12.1), **5b** (14.2), **6b** (14.3), **7b** (1.2)]. The highest SI value calculated was 63.1 for the compound **5b** toward HSC-3. Among others, compound **1b** did not show remarkable tumor specificity against cancer cells.

The most important problem in anticancer drugs is selective cytotoxicity. In this study, tumor selectivity (TS) was calculated by 2 equations as TS_1 and TS_2. First TS calculation was made by dividing the average CC_{50} value toward normal cells to the average CC_{50} value toward cancer cell lines (TS_1 = Column D/Column B, Table 1). Second TS values were also generated by considering the fact that HGF is the corresponding normal cell of Ca9-22 cancer cell line having the same origin (both derived from gingival tissues). Second TS values were generated for a compound by dividing the CC_{50} value toward HGF cells into the CC_{50} value toward Ca9-22 cell line (TS_2 = Column C/Column A., Table 1). It is clear that some compounds tested had more selectivity than 5-FU according to the first type TS_1 calculation. The highest TS value was calculated 16.4 for the compound **4b**, which has

TABLE 1 Cytotoxicities of the compounds **1b-7b**

CC_{50} (μM)																
	Human oral squamous cell carcinoma cell line								Human normal oral cells				TS		PSE	
	Ca9-22		HSC-2		HSC-3		HSC-4									
	A	SI		SI		SI		SI	mean B	HGF C	HPLF	HPC	mean D	TS_1 D/B	TS_2 C/A	$(D/B^2)\times100$	$(C/A^2)\times100$
1b	400.0	0.7	400.0	0.7	400.0	0.7	400.0	0.7	400.0	400.0	30.6	400.0	276.9	0.7	1.0	0.2	0.3
2b	15.3	6.3	24.6	3.9	27.3	3.5	37.3	2.6	26.1	253.3	10.9	22.6	95.6	3.7	16.6	14.0	108.7
3b	400.0	1.0	400.0	1.0	400.0	1.0	400.0	1.0	400.0	400.0	400.0	400.0	400.0	1.0	1.0	0.3	0.3
4b	23.0	13.8	19.3	16.5	16.2	19.5	26.2	12.1	21.2	385.3	400.0	166.3	317.2	15.0	16.8	70.7	72.8
5b	156.0	1.2	352.0	0.5	3.1	61.3	13.4	14.2	131.1	400.0	163.7	6.3	190.0	1.4	2.6	1.1	1.6
6b	10.8	28.5	26.0	11.8	16.5	18.6	21.4	14.3	18.7	315.7	301.7	302.3	306.6	16.4	29.3	88.0	272.3
7b	200.0	2.0	391.7	1.0	131.0	3.0	317.0	1.2	259.9	400.0	385.7	400.0	395.2	1.5	2.0	0.6	1.0
5-FU	22.0	44.8	261.0	3.8	7.8	126.3	12.5	78.7	75.8	1000.0	1000.0	958.3	986.1	13.0	45.4	17.1	206.0

Abbreviations: 5-FU, 5-Fluorouracil; CC_{50}, 50% cytotoxic concentration; HGF, Human gingival fibroblast; HPC, Human pulp cells; HPLF, Human periodontal ligament fibroblast; PSE, Potency-selectivity expression; TS, Tumor-selectivity index.

Note: Ca9-22, derived from gingival tissue; HSC-2, HSC-3 and HSC-4, derived from tongue.

4-methoxy-3-hydroxyphenyl ring. The bioisosteric modification of the compound **1b** (phenyl, 0.7) caused to 2 times increasing of TS value for **7b** (thiophene-2-yl, 1.5).

On the other hand, TS_2 pointed out that all of the compounds had less selectivity than 5-FU. In terms of TS, mono and tri methoxy substitution/s of phenyl ring were considered a favorable molecular modification. The compound **2b** (2-methoxy phenyl), compound **4b** (4-methoxy-3-hydroxy phenyl) and compound **6b** (3,4,5-trimethoxyphenyl) showed the best TS. TS values were 16.6, 16.8, 29.3 for **2b**, **4b**, and **6b**, respectively.

As another parameter, PSE (Potential Selectivity Expression) values were calculated by the following Equations $(D/B^2) \times 100$ and $(C/A^2) \times 100$. The compounds **4b** (with 4-methoxy-3-hydroxy phenyl, PSE = 70.7) and **6b** (with trimethoxyphenyl, PSE = 88) showed higher PSE values than 5-FU although other compounds PSE values were much lower than 5-FU (PSE = 17.1) (Table 1). When the PSE values of the compounds were compared to the non-substituted compound **1b**, it was observed that the PSE values of the substituted compounds increased compared to the non-substituted compound **1b** as follows as compound and (times of): **2b** (362), **4b** (242), **5b** (5), **6b** (906). However, when non-substituted compound **1b** was compared to compound **7b**, which is a bioisoster of compound **1b**, PSE value of compound **7b** did not increase. These kinds of compounds can be considered as the most promising compounds with good potency and selective cytotoxicity based on the PSE values.

Based on PSE and TS values, compound **6b** (2-(3-(4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-4,5-di-32#hydro-1*H*-pyrazol-1-yl)benzo[*d*]thiazole) showed remarkable cytotoxicity toward OSCC.

Carbonic anhydrase (CA) isoenzymes have become an important target in developing new inhibitor and activator drugs due to their various pharmacological effects.[21] In this study, hCA I and hCA II isoenzymes were used to identify whether the compounds have CAs inhibitory potency. According to the results obtained (Table 2), the compounds (**1b-7b**) had hCA I inhibitory potency with Ki value of 36.87 ± 11.62 – 66.24 ± 2.99 μM. Among the series, compound **1b (Ki** = 36.87 ± 11.62 μM**)** showed great inhibition properties against hCA I when compared with AZA (30.18 ± 7.77 μM).

Furthermore, compounds (**1b-7b**) had inhibition potency against hCA II with Ki values between 22.66 ± 1.41 and 89.95 ± 6.25 μM while AZA had Ki value of 4.41 $\pm$ 0.55 μM. Compound **6b**, 2-(3-(4-methoxyphenyl)-5-(3,-4,5-trimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzo [*d*]thiazole may be evaluated as the strongest compound with the lowest Ki value (22.66 ± 1.41 μM) against hCA II.

TABLE 2 hCA I and hCA II inhibition results of the compounds **1b-7b**

	IC_{50} (μM)				Ki (μM)	
Compounds	**hCA I**	r^2	**hCA II**	r^2	**hCA I**	**hCA II**
1b	28.5	0.9546	45.39	0.949	36.87 ± 11.62	57.36 ± 2.39
2b	46.5	0.9955	29.49	0.943	47.06 ± 16.89	55.57 ± 3.30
3b	20	0.9498	33.16	0.969	45.55 ± 5.37	89.95 ± 6.25
4b	21.3	0.9345	32.25	0.946	43.132 ± 4.12	25.95 ± 7.46
5b	26.5	0.9385	37.46	0.947	46.56 ± 3.45	26.72 ± 8.88
6b	27.3	0.9674	29.24	0.926	46.61 ± 2.15	22.66 ± 1.41
7b	24.3	0.9787	38.08	0.921	66.24 ± 2.99	36.57 ± 10.48
AZA*	16.6	0.9887	8.37	0.983	30.18 ± 7.77	4.41 ± 0.55

Note: *Acetazolamide (AZA) was used as a standard inhibitor for both hCA I and hCA II isoenzymes.

The compounds studied here had methoxy substituent/s (mono, di and, tri methoxy) in different positions of the phenyl ring. These modifications were provided by using benzaldehyde and mono, di and tri substituted methoxy benzaldehydes [2-methoxybenzaldehyde (**2b**), 4-methoxybenzaldehyde (**3b**), 4-methoxy-3-hydroxybenzaldehyde (**4b**), 2,5-dimethoxybenzaldehyde (**5b**) and 3,4,5-trimethoxy benzaldehyde (**6b**)] to investigate how methoxy groups affect the bioactivities (Scheme 1).

As a favorable molecular modification, the replacement of hydrogen by di and tri methoxy groups can be considered for future studies. The compound **4b** (Ki = 43.132 ± 4.12 μM, 4-methoxy-3-hydroxy derivative) against hCA I and the compound **6b** (Ki = 22.66 ± 1.41 μM, trimethoxy derivative) against hCA II were found promising CAs inhibitors.

It can be expressed here that the substitution of methoxy groups on phenyl ring affects compounds' CA inhibitory effects against hCA I/II. So, the different chemical structure of the compounds which may affect interaction with the active side may change their bioactivity.

3 | CONCLUSION

This research reported synthesis and bioactivities of the *2*-(3-(4-methoxyphenyl)-5-(aryl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzo[*d*]thiazoles. The bioactivity assays showed that the compounds (*2*-(3-(4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1 yl) benzo [*d*]thiazole) **6b** (PSE = 272.3, TS = 29.3) as cytotoxic agent and (2-(3-(4-methoxyphenyl)-5-phenyl-4,-5-dihydro-1*H*-pyrazol-1-yl)benzo[*d*]thiazole) **1b** (Ki = 36.87 ± 11.62 μM, hCA I) and **6b** (Ki = 22.66 ± 1.41 μM, hCA II) as carbonic anhydrase inhibitors can be considered for further molecular modifications and new projects.

4 | MATERIALS AND METHODS

4.1 | Chemistry

The synthesis methods of the compounds were presented in Scheme 1. During the synthesis studies, to monitor the reaction and check the purity of the synthesized compounds, thin layer chromatography (TLC) plates (60 HF254, Merck KGaA) were used. Chloroform: methanol (4.8:0.2) system was used as the mobile phase system. NMR spectra of the compounds were taken with the Varian Mercury Plus spectrometer (Varian inc., Palo Alto, California, U.S.). Shimadzu's LCMS-TOF-ESI (Shimadzu, Kyoto, Japan) device was used for HRMS. Electrothermal 9100/IA9100 instrument (Bibby Scientific Limited, Staffordshire, UK) device was used to determine melting points.

4.1.1 | Synthesis of 2-hydrazinylbenzo[*d*] thiazole (Compound A)

The starting compounds, 2-mercaptobenzothiazole (3.0g) and hydrazine hydrate (10 mL / 80%), were refluxed for 24 hours by the conventional method in ethanol (20 mL). At the end of the specified period, the contents of the flask were kept at room temperature and the separated product was filtered and dried. Light brown compound A was used for the next reaction without purification.[22]

4.1.2 | General procedure for the preparation of chalcones, 1a-7a

Starting compounds of the series, which are chalcones, were synthesized by Claisen-Schmidt condensation.[20–28] 4-Methoxy acetophenone and the appropriate aldehyde derivative [benzaldehyde (**1a**), 2-methoxybenzaldehyde

(**2a**), 4-methoxybenzaldehyde (**3a**), 3-hydroxy-4-methoxybenzaldehyde (**4a**), 2,5-dimethoxybenzaldehyde (**5a**), 3,4,5-trimethoxybenzaldehyde (**6a**), thiophene-2-carboxaldehyde (**7a**)] were mixed within ethyl alcohol (6 mL) in 1:1 mol ratio. The mixture was cooled on ice bath and then NaOH (aqua, 6 mL, 10%) was added drop by drop to the flask. The mixture was sustained at room temperature throughout the night. After 24 hours, the content was taken into the cold water (50 mL). The content of the flask was acidified with concentrated HCl acid (pH = 6-7). The collapsed solid was filtered. Water and ethanol were used to wash the solid compound. After the drying, the compound was used as a starting material in the third step.

4.1.3 | Synthesis of the pyrazoline derivatives, 1b-7b

Synthesis of pyrazoline derivative compounds (**1b-7b**) was carried out in acidic medium using a conventional method with a protic solvent. Briefly, favorable chalcone derivative (1 mmol) (**1a-7a**) and 2-hydrazinylbenzo[*d*]thiazole (1.1 mmol, compound A) in ethanol (25 mL) with acetic acid (0.05 mL) were heated for 19 - 36 hours (for **1b-7b**). Then, ethanol was evaporated until half volume and the flask was left at room temperature. After the collapsed solid was filtered, it was purified by crystallization from the favorable solvent or solvent mixture (methanol or methanol-ether).[12,29,30]

*2-(3-[4-Methoxyphenyl]-5-phenyl-4,5-dihydro-1*H*-pyrazol-1-yl)benzo[*d*]thiazole **(1b)***

A bright green solid, yield 9.3%. Mp: 188°C–190°C; Lit m. p: 149°C–150°C.[27] ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.71 (*d*, 2H, ArH, J = 8.8 Hz), 7.63 (*d*, 1H, ArH, J = 7.7 Hz), 7.50 (*d*, 1H, ArH, J = 8.1 Hz), 7.46–7.22 (m, 6H, ArH), 7.07 (t, 1H, ArH, J = 7.5 Hz), 6.94 (*d*, 2H, ArH, J = 8.8 Hz), 5.78 (dd, 1H, pyrazoline ring, J = 12.1, 5.1 Hz), 3.84 (s, 3H, OCH_3), 3.27 (dd, 1H, pyrazoline ring, J = 17.2, 5.1 Hz), (one of the proton peak of the pyrazoline ring was under methoxy peak). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 152.9, 152.7, 141.6, 131.9, 129.1, 128.7, 128.4, 127.9, 126.2, 125.8, 124.1, 121.8, 121.4, 120.9, 120.2, 114.4, 63.7, 55.6, 44.1, HRMS (ESI-MS) calc. For $C_{23}H_{19}N_3OS$ $[M + H]^+$ 386.1322; found 386.1336.

*2-(5-(2-Methoxyphenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzo[*d*]thiazole **(2b)***

A bright yellow solid, yield 39%. Mp: 199°C - 200°C. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.71 (*d*, 2H, ArH, J = 9.1 Hz), 7.66 (*d*, 1H, ArH, J = 8.1 Hz), 7.52 (*d*, 1H, ArH, J = 7.7 Hz), 7.27 - 7.21 (m, 2H, ArH), 7.15 (*d*, 1H, ArH, J = 7.7 Hz), 7.08 (t, 1H, ArH, J = 7.7 Hz), 6.93 (*d*, 3H, ArH, J = 7.3 Hz), 6.85 (t, 1H, ArH, J = 7.5 Hz), 6.03 (dd, 1H, pyrazoline ring, J = 11.7, 5.1 Hz), 3.90 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.16 (dd, 1H, pyrazoline ring, J = 17.6, 5.1 Hz), (one of the proton peak of the pyrazoline ring was under methoxy peak). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 163.6, 161.3, 156.5, 153.7, 153.1, 132.0, 128.98, 128.88, 128.3, 126.5, 125.8, 124.4, 121.7, 120.9, 120.8, 120.1, 114.3, 110.9, 59.6, 55.7, 55.6, 43.1, HRMS (ESI-MS) calc. For $C_{24}H_{21}N_3O_2S$ $[M + H]^+$ 416.1427; found 416.1435.

*2-(3,5-bis[4-Methoxyphenyl]-4,5-dihydro-1*H*-pyrazol-1-yl)benzo[*d*]thiazole **(3b)***

A bright yellow solid, yield 6.5%. Mp: 174°C–175°C. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.71 (*d*, 2H, ArH, J = 7.3 Hz), 7.62 (*d*, 1H, ArH, J = 8.1 Hz), 7.51 (*d*, 1H, ArH, J = 8.1 Hz), 7.28–7.21 (m, 3H, ArH), 7.06 (t, 1H, ArH, J = 7.5 Hz), 6.94 (*d*, 2H, ArH, J = 7.3 Hz), 6.83 (*d*, 2H, ArH, J = 7.3 Hz), 5.72 (dd, 1H, pyrazoline ring, J = 11.7, 4.8 Hz), 3.85 (s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.26 (dd, 1H, pyrazoline ring, J = 17.2, 5.1 Hz), (one of the proton peak of the pyrazoline ring was under methoxy peak). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 163.6, 161.4, 159.3, 152.9, 152.7, 133.7, 131.9, 128.3, 127.6, 125.8, 124.2, 121.8, 120.9, 120.2, 114.42, 114.38, 63.3, 55.6, 55.5, 44.1, HRMS (ESI-MS) calc. For $C_{24}H_{21}N_3O_2S$ $[M + H]^+$ 416.1427; found 416.1438.

*5-(1-(Benzo[*d*]thiazol-2-yl)-3-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-5-yl)-2-methoxyphenol **(4b)***

A cream colour solid, yield 3.3%. Mp: 225°C - 226°C. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 9.03 (s, 1H, OH), 7.76–7.69 (m, 3H, ArH), 7.37 (*d*, 1H, ArH, J = 7.7 Hz), 7.23–7.19 (m, 1H, ArH), 7.07 - 7.00 (m, 3H, ArH), 6.83 (*d*, 1H, ArH, J = 8.8 Hz), 6.67-6.50 (m, 2H, ArH), 5.61 (dd, 1H, pyrazoline ring, J = 11.3, 4.4 Hz), 3.95 (dd, 1H, pyrazoline ring, J = 17.6, 11.7 Hz), 3.78 (s, 3H, OCH_3), 3.67 (s, 3H, OCH_3), 3.22 (dd, 1H, pyrazoline ring, J = 17.9, 4.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 163.2, 161.6, 154.5, 152.9, 147.7, 147.3, 134.9, 131.6, 128.9, 126.5, 123.9, 122.3, 121.9, 119.9, 117.2, 115.0, 113.3, 113.0, 63.3, 56.2, 56.0, 44.4, HRMS (ESI-MS) calc. For $C_{24}H_{21}N_3O_3S$ $[M + H]^+$ 432.1376; found 432.1363.

*2-(5-(2,5-Dimethoxyphenyl)-3-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzo[*d*]thiazole **(5b)***

A cream colour solid, yield 10.7%. Mp: 210°C - 212°C. ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 7.71 - 7.69 (m, 2H, ArH), 7.64 (*d*, 1H, ArH, J = 7.7 Hz), 7.51 (*d*, 1H, ArH, J = 8.1 Hz), 7.26 - 7.22 (m, 1H, ArH), 7.10 - 7.06 (m, 1H, ArH), 6.93 - 6.91 (m, 2H, ArH), 6.85 - 6.83 (m, 1H, ArH), 6.75 - 6.72 (m, 2H, ArH), 5.99 (dd, 1H, pyrazoline ring,

J = 11.7, 5.1 Hz), 3.87 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 3.64 (s, 3H, OCH_3), 3.16 (dd, 1H, pyrazoline ring, J = 17.2, 5.1 Hz), (one of the proton peak of the pyrazoline ring was under methoxy peak). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 163.7, 161.3, 153.9, 153.6, 153.0, 150.7, 132.0, 130.4, 128.4, 125.7, 124.3, 121.7, 120.9, 120.2, 114.3, 113.0, 112.8, 112.1, 59.5, 56.3, 55.9, 55.6, 43.2, HRMS (ESI-MS) calc. For $C_{25}H_{23}N_3O_3S$ $[M + H]^+$ 446.1533; found 446.1527.

*2-(3-(4-Methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzo[*d*]thiazole* ***(6b)***

A cream colour solid, yield 7.2%. Mp: 182°C–183°C. ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 7.71 (*d*, 1H, ArH, J = 8.8 Hz), 7.64 (*d*, 1H, ArH, J = 8.1 Hz), 7.53 (*d*, 1H, ArH, J = 7.7 Hz), 7.28 - 7.24 (m, 2H, ArH), 7.11–7.07 (m, 1H, ArH), 6.94 (*d*, 2H, ArH, J = 8.8 Hz), 6.56 (s, 2H, ArH), 5.69 (dd, 1H, pyrazoline ring, J = 12.1, 5.5 Hz), 3.85 (s, 3H, OCH_3), 3.80 (s, 9H, OCH_3), 3.28 (dd, 1H, pyrazoline ring, J = 17.2, 5.1 Hz), (one of the proton peak of the pyrazoline ring was under methoxy peak). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 163.8, 161.5, 153.9, 153.8, 152.9, 152.8, 137.3, 131.9, 128.4, 125.9, 124.0, 121.9, 120.9, 120.2, 114.4, 114.1, 64.0, 60.9, 56.4, 56.3, 55.6, 44.2, HRMS (ESI-MS) calc. For $C_{26}H_{25}N_3O_4S$ $[M + H]^+$ 476.1639; found 476.1635.

*2-(3-(4-Methoxyphenyl)-5-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzo[*d*]thiazole* ***(7b)***

A bright yellow solid, yield 9%. Mp: 167°C–168°C. ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 7.72 (*d*, 2H, ArH, J = 8.8 Hz), 7.65 (*d*, 1H, ArH, J = 7.7 Hz), 7.59 (*d*, 1H, ArH, J = 7.7 Hz), 7.31–7.25 (m, 2H, ArH), 7.18–7.08 (m, 3H, ArH), 6.96–6.91 (m, 2H, ArH), 6.05 (dd, 1H, pyrazoline ring, J = 11.7, 4.8 Hz), 3.85 (s, 3H, OCH_3), 3.44 (dd, 1H, pyrazoline ring, J = 17.6, 4.8 Hz), (one of the proton peak of the pyrazoline ring was under methoxy peak). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 163.7, 161.5, 152.80, 152.76, 144.0, 132.0, 128.4, 127.1, 125.9, 125.8, 125.1, 123.9, 122.1, 121.0, 120.3, 114.4, 59.5, 55.6, 43.9, HRMS (ESI-MS) calc. For $C_{21}H_{17}N_3OS_2$ $[M + H]^+$ 392.0886; found 392.0895.

4.2 | Biological assays

4.2.1 | Cytotoxicity evaluation

Human oral squamous cell carcinoma cell lines (Ca9-22, HSC-2, HSC-3, HSC-4) and human normal oral cells [gingival fibroblast (HGF), periodontal ligament fibroblast (HPLF), and pulp cell (HPC)] were treated with the compounds **1b-7b** to see their cytotoxic activity with some minor modifications.[23,24,26,28,31,32] The viable cell numbers were determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. The CC_{50} values were determined from dose-response curves. 5-Fluorouracil was used as a reference compound.

4.2.2 | Carbonic anhydrase enzyme assay

Purification of cytosolic CA enzymes was carried out according to the literature.[33] Activities of these enzymes were designated according to a procedure by Verpoorte et al[34] at 348 nm. In addition, the amount of protein was designated at 595 nm.

According to the Bradford method[35], inhibition effect of **1b-7b** on both hCA enzymes, an activity (%)-[**1b-7b**] graph, was drawn. The IC_{50} values were obtained from activity (%) vs compounds plots. Ki values were calculated in three different concentrations and Lineweaver-Burk curves were drawn.[36] Acetazolamide was used as a reference compound.

ACKNOWLEDGEMENTS

The authors thank TUBITAK for financial support (Project Number: 117S939) and Ataturk University (Erzurum, Turkey) for NMR spectra.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

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How to cite this article: Tugrak M, Gul HI, Sakagami H, Gulcin I. Synthesis, cytotoxic, and carbonic anhydrase inhibitory effects of new 2-(3-(4-methoxyphenyl)-5-(aryl)-4,5-dihydro-1*H*-pyrazol-1-yl)benzo[*d*]thiazole derivatives. *J Heterocyclic Chem*. 2020;1–7. https://doi.org/10.1002/jhet.3985