ACS OMEGA

http://pubs.acs.org/journal/acsodf

Article

(Benzylideneamino)triazole−Thione Derivatives of Flurbiprofen: An Efficient Microwave-Assisted Synthesis and *In Vivo* Analgesic Potential

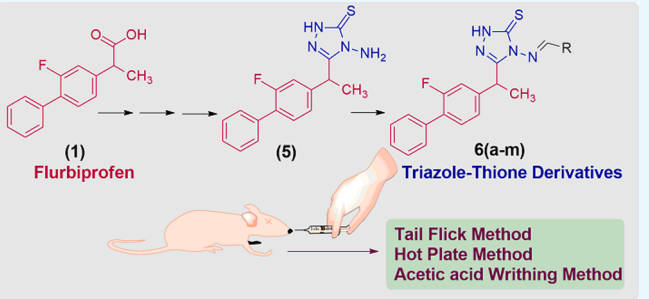
Muhammad Zaheer, Muhammad Zia-ur-Rehman,* Rubina Munir,* Nadia Jamil, Saiqa Ishtiaq, Rahman Shah Zaib Saleem, and Mark R. J. Elsegood

Cite This: *ACS Omega* 2021, 6, 31348−31357

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Triazole is an imperative heterocycle renowned for its broad-spectrum biological significance. In this manuscript, facile microwave-assisted synthesis of a series of 4-(benzylideneamino)-3-(1-(2-fluoro-[1,1′-biphenyl]-4-yl)ethyl)-1*H*-1,2,4-triazole-5(4*H*)-thione **6**(**a**−**m**) derivatives along with their *in vivo* analgesic activity is reported. 2-(2-Fluoro-[1,1′-biphenyl]-4-yl)propanoic acid (flurbiprofen) was converted to methyl 2-(2-fluoro-[1,1′-biphenyl]-4-yl)propanoate using microwave irradiation, followed by its hydrazinolysis with hydrazine monohydrate. 2-(2-Fluoro-[1,1′-biphenyl]-4-yl)propanehydrazide thus obtained was converted to 4-amino-3-(1-(2-fluoro-[1,1′-biphenyl]-4-yl)ethyl)-1*H*-1,2,4-triazole-5(4*H*)-thione, followed by its condensation with different aromatic aldehydes to get the title compounds. Structures of all the synthesized compounds were established using different methods (^{1}H NMR and ^{13}C NMR spectroscopies, mass spectrometry, and elemental analysis) and evaluated for their potential as analgesic agents by tail flick, hot plate, and writhing methods. The results of this *in vivo* study revealed several compounds as potent analgesic agents among which compound **6e** showed significant analgesic effect for all the three assays employed.



1. INTRODUCTION

Synthesis of triazoles and their biological importance have received considerable attention in recent years, and this class of compounds have become one of the potential agents for drug discovery.[1−5] Among these, 1,2,4-triazoles represent an important class of organic compounds with wide use in medicine, agriculture, and industry.[6] A wide variety of therapeutically interesting drug candidates possessing analgesic, anti-inflammatory, anti-microbial, anti-cancer, anti-depressant, anti-fungal, anti-convulsant, and anti-viral activities are discussed in the literature possessing the 1,2,4-triazole moiety.[7−16]

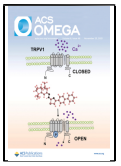
Many strategies have been reported in the literature regarding the development of more effective NSAIDs (non-steroidal anti-inflammatory drugs) with reduced side effects.[17−19] In certain cases, derivatization of the carboxylic functional group in NSAIDs led to increased analgesic and anti-inflammatory activities with reduced ulcerogenic potential.[20,21]

Flurbiprofen, 2-(2-fluoro-[1,1′-biphenyl]-4-yl)propanoic acid, is a non-selective cyclooxygenase (COX) inhibitor for the treatment of non-infectious inflammation, arthritis, and pain.[22−24] However, like other drugs of this category, it is also associated with gastro-intestinal (GI) ulceration, nephrotoxicity, and bleeding.[25,26] Keeping in view the analgesic activities of flurbiprofen and 1,2,4-triazoles, both the moieties are synergized in one hybrid unit as part of our ongoing program regarding the development of biologically active heterocycles. Flurbiprofen was esterified, followed by its hydrazinolysis, and subsequent reactions with carbon disulfide and hydrazine hydrate resulted in 4-amino-3-(1-(2-fluoro-[1,1′-biphenyl]-4-yl)ethyl)-1*H*-1,2,4-triazole-5(4*H*)-thione (**5**). It was further reacted with different aldehydes to get a series of 4-(benzylideneamino)-3-(1-(2-fluoro-[1,1′-biphenyl]-4-yl)-ethyl)-1*H*-1,2,4-triazole-5(4*H*)-thiones **6**(**a**−**m**) (Scheme 1). Besides chemical characterization, these compounds were checked for their analgesic activity on albino mice using tail flick, hot plate, and writhing methods.

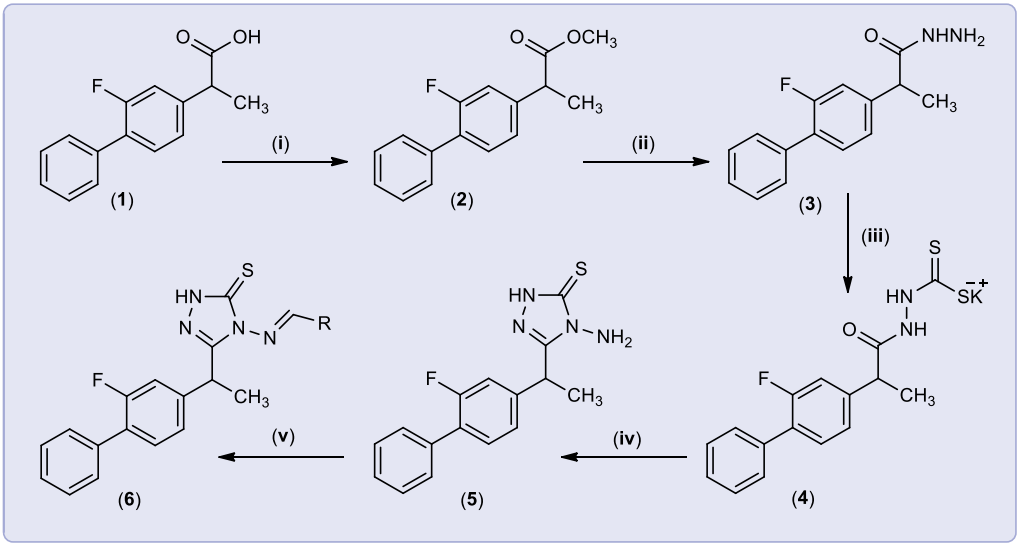
2. RESULTS AND DISCUSSION

2.1. Chemistry. 4-Amino-3-(1-(2-fluoro-[1,1′-biphenyl]-4-yl)ethyl)-1*H*-1,2,4-triazole-5(4*H*)-thione (**5**), the main inter-

Received: September 20, 2021
Accepted: October 28, 2021
Published: November 12, 2021



Scheme 1. Synthesis of 4-(Benzylideneamino)-3-(1-(2-fluoro-[1,1′-biphenyl]-4-yl)ethyl)-1*H*-1,2,4-triazole-5(4*H*)-thiones[a]



[a]Reaction conditions: (i) MeOH/H$^+$/microwaves (ii) hydrazine hydrate/MeOH/microwaves (iii) EtOH/KOH/CS_2 (iv) EtOH/hydrazine hydrate/microwaves (v) RCHO/EtOH/H$^+$/microwaves.

Table 1. Synthesis of 4-(Benzylideneamino)-3-(1-(2-fluoro-[1,1′-biphenyl]-4-yl)ethyl)-1*H*-1,2,4-triazole-5(4*H*)-thiones (6a−6n) under Conventional and Microwave Conditions

				conventional		microwave		
entry	product	*R*	M.P (°C)	reaction time (min)	yield (%)[a]	reaction time (min)	yield (%)[a]	reaction temperature (°C)
1	**6a**	phenyl	213	270	77	11	93	287
2	**6b**	2-chlorophenyl	189	269	67	15	95	330
3	**6c**	3-chlorophenyl	190	236	65	13	90	305
4	**6d**	4-chlorophenyl	196	220	73	9	97	308
5	**6e**	2-hydroxyphenyl	195	290	66	18	89	303
6	**6f**	4-hydroxyphenyl	180	280	73	14	95	291
7	**6g**	2,4-dichlorophenyl	204	210	63	20	96	317
8	**6h**	2,3-dichlorophenyl	226	240	59	23	92	320
9	**6i**	4-nitrophenyl	208	200	79	17	94	300
10	**6j**	4-N,N-dimethylphenyl	210	260	74	12	90	328
11	**6k**	4-fluorophenyl	179	200	78	20	96	308
12	**6l**	3,4-dimethoxyphenyl	170	229	71	19	94	302
13	**6m**	2-furfural	197	190	75	10	97	280

[a]Isolated yields based on 4-amino-3-(1-(2-fluoro-[1,1′-biphenyl]-4-yl)ethyl)-1*H*-1,2,4-triazole-5(4*H*)-thione.

mediate of the designed scheme, was synthesized using the multi-step synthesis strategy. Esterification of 2-(2-fluoro-[1,1′-biphenyl]-4-yl)propanoic acid (**1**) using microwaves followed by hydrazinolysis yielded 2-(2-fluoro-[1,1′-biphenyl]-4-yl)-propanehydrazide (**3**).[27] The use of microwaves in the esterification and hydrazinolysis steps plays a significant role as it assists in minimizing the reaction time and increases the yield of reactions.[28,29] During this study, the reaction yield improved from 82 to 98.81% for the esterification step, while 74.94 to 95.12% for the hydrazinolysis reaction. 2-(2-Fluoro-[1,1′-biphenyl]-4-yl)propanehydrazide (**3**) was converted to potassium 2-(2-(2-fluoro-[1,1′-biphenyl]-4-yl)propanoyl)-hydrazinecarbodithioate (**4**) with the reaction of carbon disulfide and potassium hydroxide, followed by its reaction with hydrazine hydrate to form 4-amino-3-(1-(2-fluoro-[1,1′-biphenyl]-4-yl)ethyl)-1*H*-1,2,4-triazole-5(4*H*)-thione (**5**), followed by its condensation with different aldehydes under microwaves to get the title compounds. The use of microwaves in the condensation reactions was found more effective to attain better product yields and shorter reaction times (Table 1).

Structures of synthesized compounds **6**(**a**−**m**) were confirmed through spectroscopic techniques and were found in agreement with the expected values. In ^{1}H NMR spectra, the NH proton of the triazole ring appeared as a singlet around 14.00 ppm, whereas the imine N═CH proton emerged as a singlet near 9.20−10.78 ppm. Methyl (CH_3) protons exhibited a doublet near 1.65 ppm and the methine (CH) proton gave a quartet around 4.40−4.65 ppm. Aromatic ring protons appeared between 6.76 and 8.34 ppm depending on their environment. In ^{13}C NMR spectra, methyl carbon (CH_3) and methine carbon (CH) appeared around 19.5 and 35.8 ppm individually. Thione carbon (C═S) was observed above 160.0 ppm and imine carbon (N═CH) near 153−154 ppm in all the synthesized derivatives **6**(**a**−**n**). FT-IR data, elemental analysis results, and mass spectra too were in accordance with the structures of all the compounds.

https://doi.org/10.1021/acsomega.1c05222
ACS Omega 2021, 6, 31348−31357

2.2. Stereochemistry and X-ray Crystallography. A single crystal of 4-(benzylideneamino)-3-(1-(2-fluoro-[1,1′-biphenyl]-4-yl)ethyl)-1*H*-1,2,4-triazole-5(4*H*)-thione (**6a**) as a representative of the title series of compounds **6**(**a**−**m**) was grown in 90% ethanol and examined by single-crystal X-ray diffraction. The data shows that the C═N bond exhibits the *E* configuration. It is evident from the crystal data that sulfur exists in the thione form instead of thiol (Figure 1). Molecules

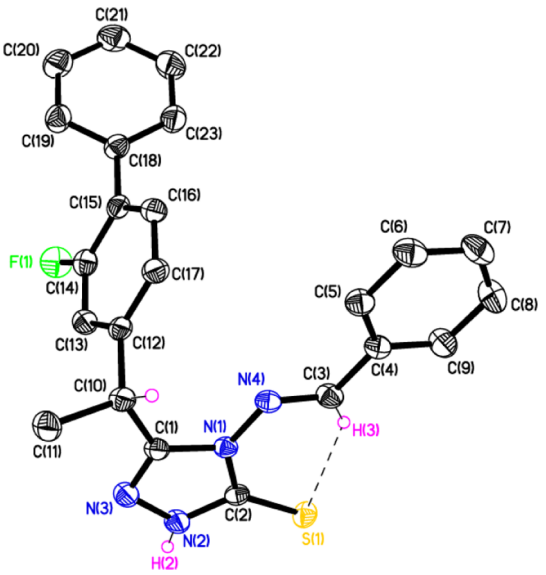


Figure 1. ORTEP diagram of compound **6a** with the numbering scheme. Displacement ellipsoids are drawn at the 50% probability level; H atoms are represented by circles of arbitrary radii.

form H-bonded dimers via pairs of centrosymmetric N−H···S interactions (Figure 2). A planar conformation between the

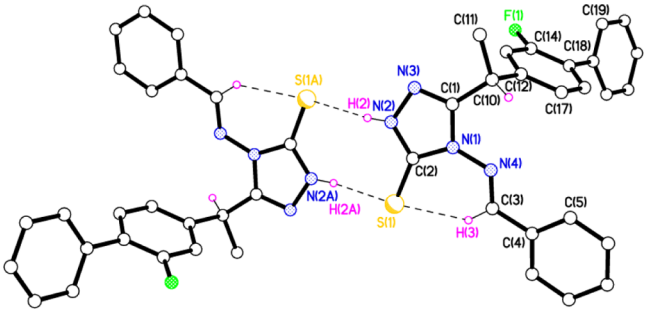


Figure 2. Perspective view showing intra- and intermolecular hydrogen bonding in **6a**.

benzylidene-amino and triazole moieties is stabilized via a C−H···S interaction. F···F interactions at 2.871 Å and C−H···F interactions give rise to an overall 3D supramolecular network. Crystal data and structure refinement details are given in the Experimental Section.

2.3. Analgesic Activity. The newly synthesized 4-(benzylideneamino)-3-(1-(2-fluoro-[1,1′-biphenyl]-4-yl)-ethyl)-1*H*-1,2,4-triazole-5(4*H*)-thiones **6**(**a**−**m**) were evaluated for their analgesic activities by three different methods, that is, tail flick, hot plate, and writhing methods. Albino mice for the current studies were housed and kept at the animal house situated at the University College of Pharmacy in the University of the Punjab Lahore, Pakistan, in groups of six and were acclimatized to experimental conditions for 2 days. Water was freely provided to the animals while access to feed was withdrawn 1 day before the experiments.

2.3.1. Tail Flick Method. Compounds **6**(**a**−**m**) were tested for their analgesic activity using the tail flick method.[30,31] This method is based on the phenomenon that certain drugs such as morphine can selectively prolong the duration of distinctive tail withdrawal reflex time when the distal end of mouse's tail is immersed in water at 55 ± 1.0 °C. Test compounds were assessed at equimolar oral doses relative to 10 mg/kg of flurbiprofen. Using this method, the analgesia effect was calculated for 0.5 to 4.5 h period since it was found that most of the animals showed analgesia ranging from 1 to 3 h. Moderate to significant activities for all the synthesized derivatives were observed, which are shown in Table 2 along with the analgesic activity of reference standard, flurbiprofen. Among the 14 tested compounds, **6e**, **6i**, **6j**, and **6m** showed highly significant analgesic activity compared with the reference drug, while compounds **6b**, **6d**, and **6g** showed significant analgesic activities. Compounds **6a**, **6f**, and **6l** showed less to moderately significant analgesic activities, while **6c**, **6h**, and **6k** showed the least activity compared with the reference drug (Figure 3).

2.3.2. Hot Plate Method. The title compounds **6**(**a**−**m**) were also evaluated for analgesic activity by using the hot plate method.[32,33] Compounds were tested at equimolar oral dose relative to 10 mg/kg flurbiprofen. The analgesia effect was checked from 0.5 to 4.5 h period, and it was found that most of the animals showed analgesia ranging from 0.5 to 3.5 h. Results showed moderate to significant analgesic activities for the test compounds **6**(**a**−**m**) and are shown in Table 3 compared with flurbiprofen (reference standard). Compounds **6e**, **6i**, **6l**, **6m**, and **6g** showed highly significant analgesic activities compared with the reference drug, while **6a**, **6h**, and **6j** exhibited moderate activities. The analgesic effects by the hot plate method are depicted in Figure 4.

2.3.3. Acetic Acid Induced Writhing Method. The analgesic activity by the acetic acid induced writhing method was studied on the title compounds.[34,35] Albino mice were kept in the test cage for half an hour for acclimatization to the environment before acetic acid injection. Animals were dosed orally (10 mg per kg body mass) with the test compounds and flurbiprofen (reference drug), and the analgesia effect was checked for the first half hour due to the fact that these compounds were found active in this time range. The percentage inhibition in the writhing method was calculated in two phases: the first phase during the initial 15 min after dose administration and the second phase for the next 15 min (16th−30th minute). Compounds **6a**, **6e**, and **6l** exhibited highly significant analgesic activities while **6b**, **6i**, **6j**, **6m**, and **6h** were found moderately active compared with the reference standard drug (Table 4). Compounds **6c**, **6d**, **6f**, **6g**, and **6k** were found to possess comparable analgesic activities to the reference drug, flurbiprofen, and this is depicted in Figure 5. All the title compounds exhibited higher activity than the standard drug flurbiprofen. It was further noted that the percentage inhibition of all the compounds increased in the second half of the trial time (from 16th minute to 30th minute) compared with the first half (1st to 15th minute).

An insight into the structure−activity relationship indicates that compounds bearing a substituent at the *ortho* or *para* positions of the phenyl ring (2-hydroxy, 4-nitro, 4-*N*,*N*-dimethylamino) attached to the triazole heterocycle are more

https://doi.org/10.1021/acsomega.1c05222
ACS Omega 2021, 6, 31348−31357

Table 2. Evaluation of the Analgesic Activity of 6(a−m) by the Tail Flick Method

	variation flicking time with ± SEM (time in sec at 55 ± 1 °C)									
compound ID	0 h	0.5 h min	1 h	1.5 h	2 h	2.5 h	3 h	3.5 h	4 h	4.5 h
flurbiprofen	2.727 ± 0.197	2.862 ± 0.262	2.917 ± 0.280	3.203 ± 0.299	3.418 ± 0.344	3.562 ± 0.516	3.432 ± 0.450	3.172 ± 0.356	2.992 ± 0.324	2.873 ± 0.286
6a	2.787 ± 0.436	3.880 ± 0.554	3.562 ± 0.401	3.313 ± 0.357	3.645 ± 0.432	3.752 ± 0.688	3.448 ± 0.799	3.113 ± 0.356	2.975 ± 0.322	2.828 ± 0.296
6b	2.415 ± 0.214	3.713 ± 0.610	4.347 ± 0.585	4.140 ± 0.795	4.383 ± 0.697	4.432 ± 0.668	4.040 ± 0.442	3.457 ± 0.358	3.097 ± 0.220	2.683 ± 0.253
6c	2.670 ± 0.311	3.817 ± 0.354	3.935 ± 0.389	3.257 ± 0.267	3.568 ± 0.544	3.385 ± 0.375	3.085 ± 0.404	2.708 ± 0.398	3.043 ± 0.358	2.930 ± 0.340
6d	2.787 ± 0.398	3.880 ± 0.506	3.562 ± 0.366	3.313 ± 0.326	3.645 ± 0.395	3.752 ± 0.628	3.448 ± 0.730	3.113 ± 0.325	2.975 ± 0.294	2.828 ± 0.270
6e	2.787 ± 0.398	4.477 ± 0.306	4.050 ± 0.429	4.640 ± 0.540	4.620 ± 0.630	3.503 ± 0.234	4.138 ± 0.382	4.050 ± 0.349	2.817 ± 0.329	2.855 ± 0.294
6f	3.828 ± 0.462	4.062 ± 0.818	3.960 ± 0.257	3.500 ± 0.270	3.907 ± 0.410	3.375 ± 0.273	3.222 ± 0.287	3.050 ± 0.186	2.682 ± 0.236	2.668 ± 0.158
6g	2.717 ± 0.213	3.340 ± 0.290	3.185 ± 0.254	4.262 ± 0.673	4.183 ± 0.519	3.132 ± 0.387	3.387 ± 0.500	3.173 ± 0.362	3.013 ± 0.295	2.867 ± 0.272
6h	1.767 ± 0.092	2.963 ± 0.262	3.375 ± 0.240	2.802 ± 0.193	3.117 ± 0.256	3.688 ± 0.335	3.853 ± 0.194	3.572 ± 0.190	2.705 ± 0.124	2.393 ± 0.130
6i	3.318 ± 0.318	3.435 ± 0.391	4.062 ± 0.560	6.078 ± 2.520	4.407 ± 0.594	3.083 ± 0.273	2.997 ± 0.268	2.915 ± 0.173	2.812 ± 0.169	2.762 ± 0.155
6j	3.880 ± 0.161	6.135 ± 0.653	6.148 ± 0.719	6.390 ± 0.749	6.265 ± 0.947	3.752 ± 0.628	3.448 ± 0.730	3.113 ± 0.325	2.975 ± 0.294	2.828 ± 0.270
6k	3.345 ± 0.170	3.948 ± 0.487	3.980 ± 0.495	4.020 ± 0.544	3.665 ± 0.581	3.752 ± 0.628	3.448 ± 0.730	3.113 ± 0.325	2.967 ± 0.292	2.845 ± 0.270
6l	3.582 ± 0.372	4.062 ± 0.818	3.960 ± 0.257	3.500 ± 0.270	3.907 ± 0.410	3.375 ± 0.273	3.202 ± 0.278	2.993 ± 0.180	2.660 ± 0.235	2.648 ± 0.158
6m	1.992 ± 0.122	5.218 ± 1.191	4.535 ± 0.797	4.163 ± 0.679	4.210 ± 0.550	5.590 ± 1.068	4.388 ± 0.453	3.097 ± 0.192	2.763 ± 0.186	2.353 ± 0.123

active as analgesic agents. Chloro and methoxy analogues exhibited significant activities regardless of their position on the phenyl ring (3-chloro, 4-chloro, 2,4-dichloro and 3,4-dimethoxy substituted derivatives), while the fluoro substituent seems to have no role in analgesic activity as the analogue came out to be least active.

3. CONCLUSIONS

Urged by the well-established analgesic properties of triazoles, a distinguished analgesic drug flurbiprofen was derivatized to obtain a series of novel 4-(benzylideneamino)-3-(1-(2-fluoro-[1,1′-biphenyl]-4-yl)ethyl)-1*H*-1,2,4-triazole-5(4*H*)-thiones **6**(**a**−**m**) by a facile microwave-assisted method in excellent yields. Facilitation of microwave irradiation during the core steps of synthesis was found reasonably effective in achieving better product yields and purities. Reaction times for the condensation reactions were significantly reduced by microwave irradiations as compared with the conventional heating method. The successful synthesis of the title compounds was validated from spectroscopic techniques and elemental analyses. The analgesic potential of the synthesized (benzylideneamino)triazole-thione derivatives of flurbiprofen was investigated *in vivo* by employing three different protocols. All the derivatives exhibited analgesic activity to a different degree; however, **6e** displayed significant analgesic potential as a result of all the three assays. These experiments revealed that the compounds under study possess prospective analgesic activities and may serve as an architype for forthcoming studies through further derivatization and/or structural alteration.

4. EXPERIMENTAL SECTION

4.1. Apparatus, Reagents, and Chemicals. Chemicals employed in the research work were purchased from Wako and E. Merck and were used as received. Solvents were purified through standard procedures before use. Fourier transform infrared (FT-IR) spectroscopy spectra were scanned on a Thermo Nicolet IR 200 spectrometer, while an LCQ Advantage Max Thermo Fisher instrument was used in the ESI mode for mass spectra. ^{1}H NMR spectra were recorded on a Bruker AVANCE-III 400 MHz spectrometer using TMS as the internal standard and ^{13}C NMR spectra were recorded at 101 MHz. Melting points were determined on a Gallenkamp instrument and are uncorrected. For microwave-assisted reactions, a customized microwave oven (Orient eNNe781JF) equipped with inverter technology (for realistic control of the microwaves) operating at multiples of 100 W up to 1000 W generating 2450 MHz frequency was used. The temperature of the microwave-assisted reactions was monitored by Redington 9975-IRT gun. Crystal data were collected on a Bruker APEX 2 CCDD diffractometer using graphite-monochromated Mo Kα radiation.

4.2. General Procedures for Synthesis. Compounds **2** and **3** were synthesized by using our reported methods.[27]

*4.2.1. 4-Amino-3-(1-(2-Fluoro-[1,1′-biphenyl]-4-yl)ethyl)-1H-1,2,4-triazole-5(4H)-thione (**5**).* A mixture of 2-(2-fluoro-[1,1′-biphenyl]-4-yl)propanehydrazide (**3**) (0.500 g; 1.93 mmol), potassium hydroxide (0.108 g, 1.93 mmol), and carbon disulfide (0.147 g, 0.117 mL: 1.93 mmol) dissolved in ethanol (25 mL) was agitated for 16 h at room temperature. Ether was added to the mixture and the precipitated potassium 2-(2-(2-fluoro-[1,1′-biphenyl]-4-yl)propanoyl)-hydrazinecarbodithioate (**4**) was collected by filtration. It was

https://doi.org/10.1021/acsomega.1c05222
ACS Omega 2021, 6, 31348−31357

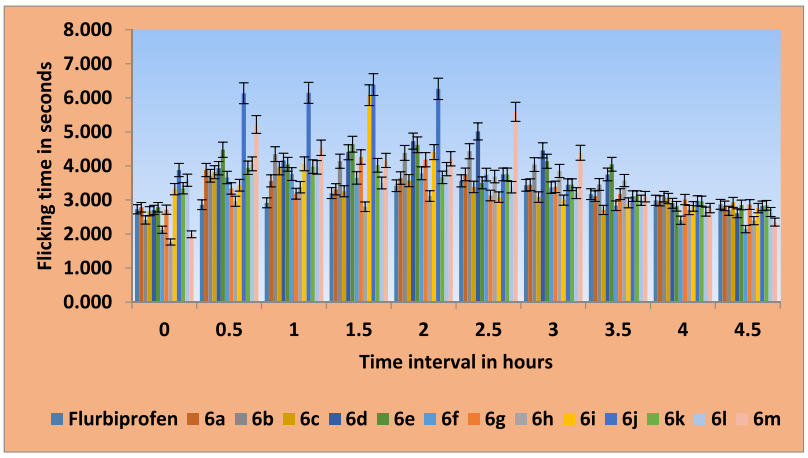


Figure 3. Analgesic activity by the tail flick method.

then washed with ether and dried under vacuum. The filtered precipitates thus obtained (0.500 g) were dissolved in hydrazine hydrate 20% (6 mL) and reacted under microwaves for 30 min until complete evolution of hydrogen sulfide. Dilute acetic acid (50 mL; 0.1%) was added to the resultant solution to get white precipitates, which were crystallized from ethanol; mp 196 °C; IR (KBr) cm^{-1}: 3314, 3140, 1265; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.58 (d, J = 7.2 Hz, 3H, CH_3), 4.44 (q, J = 7.2 Hz, 1H, CH), 5.48 (s, 2H, NH_2), 7.17−7.26 (m, 2H, ArH), 7.32−7.42 (m, 2H, ArH), 7.45−7.56 (m, 4H, ArH), 13.65 (s, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6, 101 MHz): δ 19.5, 34.9, 115.3 (d, J = 23.2 Hz), 123.9 (d, J = 3.0 Hz), 126.8 (d, J = 14.1 Hz), 127.9, 128.7, 128.8 (d, J = 3.0 Hz), 130.9 (d, J = 4.0 Hz), 135.0, 143.4 (d, J = 8.1 Hz), 154.2, 159.0 (d, J = 246.4 Hz), 166.4 ppm; Anal. Calcd for $C_{16}H_{15}FN_4S$: C, 61.13; H, 4.81; N, 17.82. Found: C, 61.11; H, 4.77; N, 17.85; MS m/z: $[M + H]^+$ 315.77.

4.2.2. General Procedure for the Synthesis of 4-(Benzylideneamino)-3-(1-(2-fluoro-[1,1′-biphenyl]-4-yl)-ethyl)-1H-1,2,4-triazole-5(4H)-thiones ***6(a−m)***. A mixture of 4-amino-3-(1-(2-fluoro-[1,1′-biphenyl]-4-yl)ethyl)-1*H*-1,2,4-triazole-5(4*H*)-thione (**5**) (200 mg; 0.774 mmol), aromatic aldehyde (0.774 mmol), ethanol (25 mL), and glacial acetic acid (1−2 drops) was irradiated to reflux under microwaves till completion of the reaction. After removal of excess ethanol under vacuum, the contents were neutralized with ice-cooled aqueous sodium bicarbonate solution (4% w/w). The products were filtered and recrystallized from alcohol.

4.2.2.1. (E)-4-(Benzylideneamino)-3-(1-(2-fluoro-[1,1′-biphenyl]-4-yl)ethyl)-1H-1,2,4-triazole-5(4H)-thione ***(6a)***. Off-white crystalline powder; mp 213 °C. IR (KBr) cm^{-1}: 3083, 1579, 1219; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.65 (d, J = 6.8 Hz, 3H, CH_3), 4.55 (q, J = 6.8 Hz, 1H, CH), 7.20 (dd, J = 8.0 Hz, 1.2 Hz, 1H, ArH), 7.26 (dd, J = 11.6 Hz, 1.2 Hz, 1H, ArH), 7.39 (t, J = 6.4 Hz, 1H, ArH), 7.44−7.62 (m, 8H, ArH), 7.83 (d, J = 7.2 Hz, 2H, ArH), 9.90 (s, 1H, N=CH), 14.02 (s, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6, 101 MHz): δ 19.6, 35.8, 115.7 (d, J = 23.2 Hz), 124.3 (d, J = 3.3 Hz), 127.2 (d, J = 13.1 Hz), 128.3, 128.9, 129.0, 129.1 (d, J = 3.0 Hz), 129.6, 131.3 (d, J = 4.0 Hz), 133.1, 132.6, 135.2, 143.7 (d, J = 8.1 Hz), 153.4, 159.3 (d, J = 247.5 Hz), 162.1, 163.6 ppm; Anal. Calcd for $C_{23}H_{19}FN_4S$: C, 68.63; H, 4.76; N, 13.92. Found: C, 68.23; H, 4.75; N, 13.89; MS m/z: $[M + H]^+$ 403.20.

4.2.2.2. 4-((2-Chlorobenzylidene)amino)-3-(1-(2-fluoro-[1,1′-biphenyl]-4-yl)ethyl)-1H-1,2,4-triazole-5(4H)-thione ***(6b)***. Yellow crystalline powder; mp 189 °C. IR (KBr) cm^{-1}: 3058, 1582, 1222; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.64 (d, J = 7.2 Hz, 3H, CH_3), 4.60 (q, J = 7.2 Hz, 1H, CH), 7.21 (dd, J = 8.0, 1.6 Hz, 1H, ArH), 7.28 (dd, J = 11.9, 1.7 Hz, 1H, ArH), 7.32−7.52 (m, 7H, ArH) 7.55−7.64 (m, 2H, ArH), 8.02 (d, J = 7.6 Hz, 1H, ArH), 10.72 (s, 1H, N=CH), 14.08 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 101 MHz): δ 19.3, 35.4, 115.3 (d, J = 23.2 Hz), 123.9, 126.9 (d, J = 14.1 Hz), 127.6, 127.9, 128.0, 128.7, 128.8 (d, J = 3.0 Hz), 130.0, 130.4, 131.0 (d, J = 3.0 Hz), 134.1, 134.8, 135.2, 143.5 (d, J = 8.1 Hz), 153.4, 156.4, 159.0 (d, J = 247.5 Hz), 161.7 ppm; Anal. Calcd for $C_{23}H_{18}ClFN_4S$: C, 63.22; H, 4.15; N, 12.82. Found: C, 63.23; H, 4.16; N, 12.79; MS m/z: $[M + H]^+$ 437.58.

4.2.2.3. 4-((3-Chlorobenzylidene)amino)-3-(1-(2-fluoro-[1,1′-biphenyl]-4-yl)ethyl)-1H-1,2,4-triazole-5(4H)-thione ***(6c)***. White powder; mp 190 °C. IR (KBr) cm^{-1}: 3152, 1562, 1239; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.64 (d, J = 6.8 Hz, 3H, CH_3), 4.57 (q, J = 7.1 Hz, 1H, CH), 7.18 (d, J = 8.0 Hz, 1H, ArH), 7.26 (d, J = 11.6 Hz, 1H, ArH), 7.36−7.46 (m, 6H, ArH), 7.56 (t, J = 7.6 Hz, 1H, ArH), 7.66 (d, J = 7.6 Hz, 1H, ArH), 7.77 (d, J = 7.6 Hz, 1H, ArH), 7.84 (s, 1H, ArH), 10.01 (s, 1H, N=CH), 14.04 (s, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6, 101 MHz): δ 19.6, 35.8, 115.7 (d, J = 23.2 Hz), 124.2 (d, J = 3.0 Hz), 127.2 (d, J = 13.1 Hz), 127.9, 128.3, 129.0, 129.1 (d, J = 3.0 Hz), 131.4 (d, J = 3.0 Hz), 131.5, 132.7, 134.4, 134.8, 135.1, 135.2, 143.8 (d, J = 8.1 Hz), 153.5, 159.4 (d, J = 247.5 Hz), 161.2, 162.2 ppm. Anal. calculated for $C_{23}H_{18}ClFN_4S$: C, 63.22 H, 4.15 N, 12.82; Found: C, 63.21; H, 4.17; N, 12.83; MS m/z: $[M + H]^+$ 437.93.

4.2.2.4. 4-((4-Chlorobenzylidene)amino)-3-(1-(2-fluoro-[1,1′-biphenyl]-4-yl)ethyl)-1H-1,2,4-triazole-5(4H)-thione ***(6d)***. Light brown powder; mp 196 °C. IR (KBr) cm^{-1}: 3085, 1554, 1220; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.64 (d, J = 6.8 Hz, 3H, CH_3), 4.58 (q, J = 7.1 Hz, 1H, CH), 7.21 (dd, J = 8.0, 1.6 Hz, 1H, ArH), 7.28 (dd, J = 11.9, 1.7 Hz, 1H, ArH), 7.39−7.48 (m, 6H, ArH), 7.84 (d, J = 7.6 Hz, 2H, ArH), 8.00 (d, J = 7.6 Hz, 2H, ArH), 10.43 (s, 1H, N=CH), 14.01 (s, 1H, NH) ppm; ^{13}C NMR (DMSO-d_6, 101 MHz): δ 19.6, 35.8, 115.7 (d, J = 23.2 Hz), 124.0 (d, J = 3.0 Hz), 124.3, 126.9 (d, J = 13.1 Hz), 127.9, 128.7, 128.8 (d, J = 3.0 Hz), 129.7, 131.1 (d, J = 3.0 Hz), 134.8, 138.1, 143.3 (d, J = 8.1 Hz), 149.5, 153.3, 159.0 (d, J = 247.5 Hz), 159.2, 163.0 ppm; Anal. Calcd for $C_{23}H_{18}ClFN_4S$: C, 63.22; H, 4.15; N, 12.82. Found: C, 63.21; H, 4.18; N, 12.83; MS m/z: $[M + H]^+$ 437.07.

https://doi.org/10.1021/acsomega.1c05222
ACS Omega 2021, 6, 31348−31357

Table 3. Evaluation of the Analgesic Activity of the Synthesized Derivatives by the Hot Plate Method

	variation of hot plate time with ± SEM (time in sec at 55 ± 1 °C)								
compound ID	0 h	0.5 h min	1 h	1.5 h	2 h	2.5 h	3 h	3.5 h	4 h	4.5 h
flurbiprofen	6.982 ± 0.763	8.312 ± 0.904	14.588 ± 1.226	15.343 ± 2.198	15.058 ± 1.125	12.925 ± 1.001	15.402 ± 0.359	13.403 ± 1.517	10.497 ± 1.199	9.152 ± 0.695
6a	9.195 ± 0.913	15.228 ± 0.866	19.473 ± 1.508	18.547 ± 1.107	18.022 ± 1.600	22.628 ± 2.135	18.412 ± 1.551	14.828 ± 1.303	11.397 ± 0.702	9.218 ± 0.682
6b	7.655 ± 0.484	15.448 ± 0.985	17.745 ± 1.353	18.062 ± 1.240	19.267 ± 2.308	23.900 ± 1.340	23.332 ± 1.136	23.337 ± 2.010	16.233 ± 1.563	12.352 ± 0.904
6c	6.758 ± 0.454	14.023 ± 1.138	18.325 ± 1.000	19.703 ± 0.600	20.838 ± 1.367	23.928 ± 1.292	22.413 ± 1.327	18.330 ± 1.078	15.718 ± 1.457	13.255 ± 0.574
6d	7.462 ± 0.878	14.195 ± 1.109	14.645 ± 0.546	16.490 ± 0.706	18.463 ± 0.744	20.095 ± 1.048	21.192 ± 1.080	19.838 ± 1.360	15.588 ± 1.180	10.742 ± 1.972
6e	8.492 ± 0.708	11.450 ± 0.594	16.188 ± 1.108	18.902 ± 1.405	21.263 ± 1.144	23.280 ± 2.092	22.110 ± 1.667	17.265 ± 1.209	12.908 ± 0.863	11.797 ± 0.600
6f	7.472 ± 0.635	11.777 ± 0.635	16.052 ± 1.060	19.058 ± 1.313	21.273 ± 1.137	23.280 ± 2.092	22.358 ± 1.681	17.217 ± 1.197	12.908 ± 0.863	11.612 ± 0.573
6g	8.913 ± 0.731	11.507 ± 0.954	13.890 ± 1.372	22.338 ± 1.908	20.625 ± 2.336	21.125 ± 1.351	27.443 ± 2.887	24.308 ± 1.899	19.338 ± 1.336	14.372 ± 0.998
6h	8.125 ± 0.427	11.607 ± 0.829	15.588 ± 0.763	18.975 ± 0.400	22.832 ± 0.700	21.763 ± 0.737	24.245 ± 1.285	20.905 ± 1.516	15.713 ± 1.117	13.935 ± 1.016
6i	8.880 ± 0.983	15.382 ± 4.119	28.280 ± 2.292	30.052 ± 2.256	24.538 ± 3.015	23.367 ± 2.004	21.077 ± 2.704	19.075 ± 2.380	15.650 ± 2.295	11.935 ± 1.012
6j	7.217 ± 0.665	12.328 ± 0.778	18.018 ± 1.652	15.967 ± 0.892	16.570 ± 1.677	16.518 ± 1.821	15.208 ± 1.107	13.400 ± 1.014	11.303 ± 0.756	10.175 ± 0.546
6k	6.395 ± 0.612	8.527 ± 0.668	15.082 ± 1.877	18.757 ± 1.917	20.225 ± 2.697	21.762 ± 2.976	17.453 ± 1.851	14.413 ± 1.149	12.145 ± 0.787	11.123 ± 0.415
6l	9.180 ± 1.293	16.405 ± 1.836	18.095 ± 1.424	21.245 ± 0.604	21.778 ± 1.462	24.965 ± 1.462	26.450 ± 2.602	22.797 ± 3.007	18.278 ± 2.195	13.438 ± 1.065
6m	7.087 ± 0.669	12.118 ± 0.895	15.845 ± 1.103	17.353 ± 1.323	18.417 ± 0.725	20.087 ± 1.026	16.632 ± 0.832	15.397 ± 1.448	11.953 ± 0.910	10.780 ± 0.807

4.2.2.5. 3-(1-(2-Fluoro-[1,1′-biphenyl]-4-yl)ethyl)-4-((2-hydroxybenzylidene)amino)-1H-1,2,4-triazole-5(4H)-thione (6e). Off-white powder; mp 195 °C. IR (KBr) cm^{-1}: 3109, 1584, 1211; ^1H NMR (DMSO-d_6, 400 MHz): δ 1.62 (d, J = 7.2 Hz, 3H, CH_3), 4.51 (q, J = 7.2 Hz, 1H, C*H*), 6.90−6.97 (m, 2H, Ar*H*), 7.16 (dd, J = 8.0, 1.6 Hz, 1H, Ar*H*), 7.23 (dd, J = 11.6, 1.6 Hz, 1H, Ar*H*), 7.37−7.49 (m, 7H, Ar*H*), 7.77 (dd, J = 7.6, 1.6 Hz, 1H, Ar*H*), 9.01 (s, 1H, O*H*), 10.03 (s, 1H, N=C*H*), 13.94 (s, 1H, N*H*) ppm; ^{13}C NMR (DMSO-d_6, 101 MHz): δ 19.3, 35.5, 115.3 (d, J = 23.2 Hz), 116.7, 118.4, 119.7, 123.8, 126.9 (d, J = 13.1 Hz), 127.2, 127.9, 128.7, 128.8 (d, J = 3.0 Hz), 131.0 (d, J = 3.0 Hz), 134.3, 134.9, 143.4 (d, J = 8.1 Hz), 152.9, 157.8, 158.5, 160.2, 161.6 ppm; Anal. Calcd for $C_{23}H_{19}FN_4OS$: C, 66.01; H, 4.58; N, 13.39. Found: C, 66.03; H, 4.59; N, 13.41; MS m/z: $[M + H]^+$ 419.23.

4.2.2.6. 3-(1-(2-Fluoro-[1,1′-biphenyl]-4-yl)ethyl)-4-((4-hydroxybenzylidene)amino)-1H-1,2,4-triazole-5(4H)-thione (6f). White powder; mp 180 °C. IR (KBr) cm^{-1}: 3142, 1575, 1210; ^1H NMR (DMSO-d_6, 400 MHz): δ 1.62 (d, J = 6.8 Hz, 3H, CH_3), 4.58 (q, J = 7.1 Hz, 1H, C*H*), 6.92 (d, J = 7.8 Hz, 2H, Ar*H*), 7.18 (dd, J = 8.0, 1.6 Hz, 1H, Ar*H*), 7.25 (dd, J = 11.6, 1.6 Hz, 1H, Ar*H*), 7.36−7.48 (m, 6H, Ar*H*), 7.58 (d, J = 7.5 Hz, 2H, Ar*H*), 9.98 (s, 1H, O*H*), 10.38 (s, 1H, N=C*H*), 14.00 (s, 1H, N*H*) ppm; ^{13}C NMR (DMSO-d_6, 101 MHz): δ 19.3, 35.5, 111.2, 115.3 (d, J = 23.2 Hz), 118.6, 123.7, 126.5 (d, J = 13.1 Hz), 127.8, 128.7, 128.8 (d, J = 3.0 Hz), 130.3, 131.0 (d, J = 3.0 Hz), 134.9, 143.3 (d, J = 7.1 Hz), 152.5, 153.7, 159.0 (d, J = 246.5 Hz), 161.7, 163.0; Anal. Calcd for $C_{23}H_{19}FN_4OS$: C, 66.01; H, 4.58; N, 13.39. Found: C, 65.99; H, 4.56; N, 13.43; MS m/z: $[M + H]^+$ 419.48.

4.2.2.7. 4-((2,4-Dichlorobenzylidene)amino)-3-(1-(2-fluoro-[1,1′-biphenyl]-4-yl)ethyl)-1H-1,2,4-triazole-5(4H)-thione (6g). Yellow powder; mp 204 °C. IR (KBr) cm^{-1}: 3094, 1536, 1212; ^1H NMR (DMSO-d_6, 400 MHz): δ 1.65 (d, J = 7.2 Hz, 3H, CH_3), 4.62 (q, J = 7.2 Hz, 1H, C*H*), 7.23 (dd, J = 8.0 Hz, 1.2 Hz, 1H, Ar*H*), 7.28 (dd, J = 12.0 Hz, 0.8 Hz, 1H, Ar*H*), 7.37−7.50 (m, 6H, Ar*H*), 7.61 (dd, J = 8.4, 1.6 Hz, 1H, Ar*H*), 7.83 (d, J = 2.0 Hz, 1H, Ar*H*), 8.05 (d, J = 8.4 Hz, 1H, Ar*H*), 10.78 (s, 1H, N=C*H*), 14.09 (s, 1H, N*H*) ppm; ^{13}C NMR (DMSO-d_6, 101 MHz): δ 19.7, 35.7, 115.6 (d, J = 24.2 Hz), 124.3 (d, J = 3.0 Hz), 127.3 (d, J = 14.1 Hz), 128.3, 128.8, 129.0, 129.1, 129.2 (d, J = 3.0 Hz), 129.5, 130.3, 131.4 (d, J = 4.0 Hz), 135.2, 136.3, 138.2, 143.8 (d, J = 8.1 Hz), 153.8, 155.3, 159.4 (d, J = 247.5 Hz), 162.0 ppm; Anal. Calcd for $C_{23}H_{17}Cl_2FN_4S$: C, 58.60; H, 3.64; N, 11.89. Found: C, 58.60; H, 3.63; N, 11.92; MS m/z: $[M + H]^+$ 472.18.

4.2.2.8. 4-((2,3-Dichlorobenzylidene)amino)-3-(1-(2-fluoro-[1,1′-biphenyl]-4-yl)ethyl)-1H-1,2,4-triazole-5(4H)-thione (6h). Off-white powder; mp 226 °C. IR (KBr) cm^{-1}: 3100, 1558, 1209; ^1H NMR (DMSO-d_6, 400 MHz): δ 1.65 (d, J = 7.2 Hz, 3H, CH_3), 4.62 (q, J = 7.2 Hz, 1H, C*H*), 7.23 (dd, J = 8.0 Hz, 1.6 Hz, 1H, Ar*H*), 7.30 (dd, J = 11.6 Hz, 1.6 Hz, 1H, Ar*H*), 7.37−7.50 (m, 6H, Ar*H*), 7.54 (t, J = 8.0 Hz, 1H, Ar*H*), 7.87 (dd, J = 8.0 Hz, 1.6 Hz, 1H, Ar*H*), 8.00 (dd, J = 8.0 Hz, 1.2 Hz, 1H, Ar*H*), 10.15 (s, 1H, N=C*H*), 10.90 (s, 1H, N*H*) ppm; ^{13}C NMR (DMSO-d_6, 101 MHz): δ 19.8, 35.7, 115.8 (d, J = 24.2 Hz), 124.3, 126.6, 127.2 (d, J = 17.1 Hz), 128.3, 129.0, 129.1 (d, J = 3.0 Hz), 129.2, 131.4, 133.0, 133.2 (d, J = 4.0 Hz), 134.3, 135.2, 143.9 (d, J = 8.1 Hz), 153.8, 155.7, 155.8, 159.3 (d, J = 247.5 Hz), 162.0 ppm; Anal. Calcd for $C_{23}H_{17}Cl_2FN_4S$: C, 58.60; H, 3.64; N, 11.89. Found: C, 58.63; H, 3.65; N, 11.92; MS m/z: $[M + H]^+$ 471.00.

https://doi.org/10.1021/acsomega.1c05222
ACS Omega 2021, 6, 31348−31357

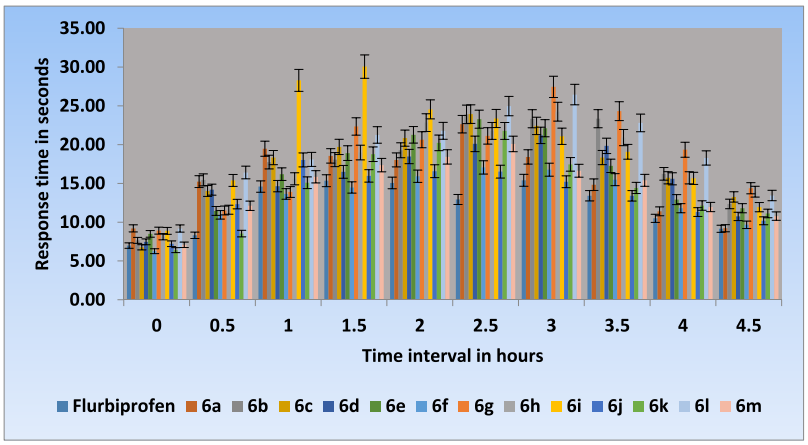


Figure 4. Analgesic activity by the hot plate method.

Table 4. Analgesic Activity by the Acetic Acid Induced Writhing Method

		mean no. of writhes ± SEM		inhibition (%)	
treatment	dose mg/kg orally	first phase	second phase	first phase	second phase
control	0.5 mL saline	57 ± 1.86	29 ± 0.951		
flurbiprofen	10 mg/kg	20.83 ± 1.249	10.83 ± 0.601	20.45	62.64
6a	10 mg/kg	17.17 ± 0.872	5.33 ± 0.760	69.88	80.25
6b	10 mg/kg	20.17 ± 1.720	6.83 ± 1.351	64.62	74.69
6c	10 mg/kg	20 ± 1.483	5.67 ± 0.881	64.91	79.01
6d	10 mg/kg	18.67 ± 1.646	5.50 ± 0.992	67.25	79.63
6e	10 mg/kg	18.33 ± 2.108	5.67 ± 0.881	67.84	79.9
6f	10 mg/kg	18 ± 1.612	5.83 ± 1.612	68.42	78.4
6g	10 mg/kg	18.33 ± 1.429	6.17 ± 1.429	67.84	77.16
6h	10 mg/kg	19 ± 2.049	6.5 ± 1.176	66.67	75.93
6i	10 mg/kg	24 ± 2.082	7.5 ± 0.992	59.65	72.22
6j	10 mg/kg	21.83 ± 1.447	6.33 ± 0.666	61.7	76.54
6k	10 mg/kg	21 ± 1.825	8.33 ± 0.881	63.16	69.14
6l	10 mg/kg	21.17 ± 2.300	6 ± 0.966	62.87	77.78
6m	10 mg/kg	22.5 ± 1.746	6.33 ± 0.881	60.53	79.54

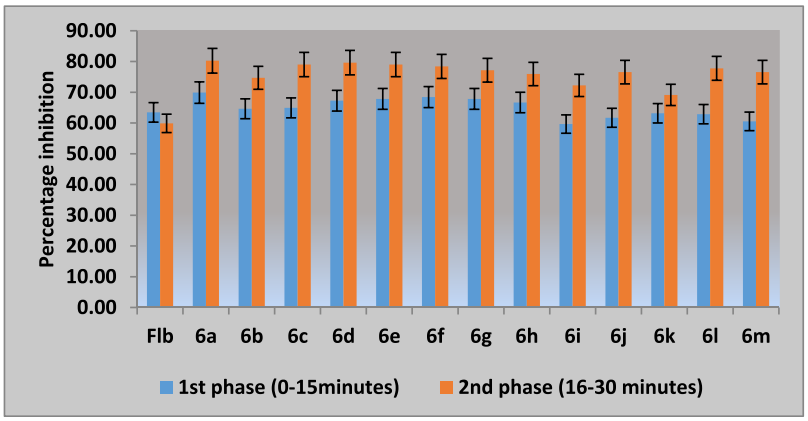


Figure 5. Analgesic activity by the acetic acid induced writhing method.

*4.2.2.9. 3-(1-(2-Fluoro-[1,1′-biphenyl]-4-yl)ethyl)-4-((4-nitrobenzylidene)amino)-1H-1,2,4-triazole-5(4H)-thione (**6i**).* Dark yellow powder; mp 208 °C. IR (KBr) cm^{-1}: 3098, 1579, 1278; ^1H NMR (DMSO-d_6, 400 MHz): δ 1.65 (d, J = 7.2 Hz, 3H, CH_3), 4.59 (q, J = 7.2 Hz, 1H, C*H*), 7.23 (dd, J = 8.0 Hz, 1.6 Hz, 1H, Ar*H*), 7.27 (dd, J = 11.6 Hz, 1.6 Hz, 1H, Ar*H*), 7.35−7.48 (m, 6H, Ar*H*), 8.09 (d, J = 8.8 Hz, 2H, Ar*H*), 8.34 (d, J = 8.8 Hz, 2H, Ar*H*), 10.32 (s, 1H, N=C*H*), 14.10 (s, 1H, N*H*) ppm; ^{13}C NMR (DMSO-d_6, 101 MHz): δ 19.3, 35.4, 115.2 (d, J = 23.2 Hz), 124.0 (d, J = 3.0 Hz), 124.3, 126.9 (d, J = 13.1 Hz), 127.9, 128.7, 128.8 (d, J = 3.0 Hz), 129.7, 131.1 (d, J = 3.0 Hz), 134.8, 138.3, 143.3 (d, J = 8.1 Hz), 149.5, 153.3, 159.0 (d, J = 247.5 Hz), 159.2, 161.8 ppm; Anal. Calcd for $C_{23}H_{18}FN_5O_2S$: C, 61.46; H, 4.48; N, 15.58. Found: C, 61.49; H, 4.49; N, 15.61; MS m/z: $[M + H]^+$ 448.11.

https://doi.org/10.1021/acsomega.1c05222
ACS Omega 2021, 6, 31348−31357

4.2.2.10. 4-((4-(Dimethylamino)benzylidene)amino)-3-(1-(2-fluoro-[1,1′-biphenyl]-4-yl)ethyl)-1H-1,2,4-triazole-5(4H)-thione (6j). Yellow-colored powder; mp 210 °C. IR (KBr) cm^{-1}: 3133, 1554, 1199; ^1H NMR (DMSO-d_6, 400 MHz): δ 1.61 (d, J = 7.2 Hz, 3H, CH_3), 3.02 (s, 6H, $N(CH_3)_2$), 4.44 (q, J = 7.2 Hz, 1H, C*H*), 6.77 (d, J = 8.8 Hz, 2H, Ar*H*), 7.15 (dd, J = 8.0 Hz, 1.6 Hz, 1H, Ar*H*), 7.20 (dd, J = 12.0 Hz, 1.2 Hz, 1H, Ar*H*), 7.36−7.48 (m, 6H, Ar*H*), 7.61 (d, J = 9.2 Hz, 2H, Ar*H*), 9.27 (s, 1H, N=C*H*), 13.84 (s, 1H, N*H*) ppm; ^{13}C NMR (DMSO-d_6, 101 MHz): δ 19.1, 35.5, 39.7, 111.6, 115.3 (d, J = 23.2 Hz), 118.7, 123.9, 126.8 (d, J = 13.1 Hz), 127.9, 128.7, 128.8 (d, J = 3.0 Hz), 130.4, 130.9 (d, J = 3.0 Hz), 134.9, 143.4 (d, J = 7.1 Hz), 152.7, 153.2, 159.0 (d, J = 246.5 Hz), 161.7, 165.0 ppm; Anal. Calcd for $C_{25}H_{24}FN_5S$: C, 67.39; H, 5.43; N, 15.72. Found: C, 67.42; H, 5.45; N, 15.73; MS m/z: $[M + H]^+$ 446.13.

4.2.2.11. 3-(1-(2-Fluoro-[1,1′-biphenyl]-4-yl)ethyl)-4-((4-fluorobenzylidene)amino)-1H-1,2,4-triazole-5(4H)-thione (6k). White powder; mp 179 °C. IR (KBr) cm^{-1}: 3176, 1511, 1236; ^1H NMR (DMSO-d_6, 400 MHz): δ 1.63 (d, J = 7.2 Hz, 3H, CH_3), 4.53 (q, J = 7.2 Hz, 1H, C*H*), 7.19 (dd, J = 8.0 Hz, 1.6 Hz, 1H, Ar*H*), 7.24 (dd, J = 12.0 Hz, 1.6 Hz, 1H, Ar*H*), 7.36−7.48 (m, 8H, Ar*H*), 7.90 (dd, J = 8.8 Hz, 5.6 Hz, 2H, Ar*H*), 9.85 (s, 1H, N=C*H*), 14.01 (s, 1H, N*H*) ppm; ^{13}C NMR (DMSO-d_6, 101 MHz): δ 19.2, 35.4, 115.3 (d, J = 24.2 Hz), 116.5 (d, J = 22.2 Hz), 124.0 (d, J = 3.0 Hz), 126.9 (d, J = 13.1 Hz), 127.9, 128.7, 128.8 (d, J = 3.0 Hz), 128.9 (d, J = 3.0 Hz), 131.0 (d, J = 4.0 Hz), 131.2 (d, J = 9.1 Hz), 134.8, 143.3 (d, J = 7.1 Hz), 153.0, 159.0 (d, J = 247.5 Hz), 161.7, 162.3, 164.8 (d, J = 252.5 Hz) ppm; Anal. Calcd for $C_{23}H_{18}F_2N_4S$: C, 65.70; H, 4.31; N, 13.32. Found: C, 65.71; H, 4.33; N, 13.35; MS m/z: $[M + H]^+$ 421.47.

4.2.2.12. 4-((3,4-Dimethoxybenzylidene)amino)-3-(1-(2-fluoro-[1,1′-biphenyl]-4-yl)ethyl)-1H-1,2,4-triazole-5(4H)-thione (6l). Brown powder; mp 170 °C. IR (KBr) cm^{-1}: 3139, 1574, 1214; ^1H NMR (DMSO-d_6, 400 MHz): δ 1.62 (d, J = 7.2 Hz, 3H, CH_3), 3.82−3.83 (2 s (merged), 6H, OCH_3), 4.51 (q, J = 7.2 Hz, 1H, C*H*), 7.07 (d, J = 8.4 Hz, 1H, Ar*H*), 7.16 (dd, J = 8.0 Hz, 1.6 Hz, 1H, Ar*H*), 7.27 (dd, J = 11.6 Hz, 1.6 Hz, 1H, Ar*H*), 7.32 (dd, J = 8.4 Hz, 2.0 Hz, 1H, Ar*H*), 7.37−7.45 (m, 7H, Ar*H*), 9.68 (s, 1H, N=C*H*), 13.94 (s, 1H, N*H*) ppm; ^{13}C NMR (DMSO-d_6, 101 MHz): δ 19.2, 35.6, 55.5, 55.8, 108.6, 111.5, 115.4 (d, J = 23.2 Hz), 123.8 (d, J = 3.0 Hz), 124.7, 126.8 (d, J = 13.1 Hz), 127.9, 128.7, 128.8 (d, J = 3.0 Hz), 131.0 (d, J = 3.0 Hz), 134.8, 143.6 (d, J = 8.1 Hz), 149.2, 152.8, 152.9, 153.3, 159.0 (d, J = 247.5 Hz), 161.7, 163.0 ppm; Anal. Calcd for $C_{25}H_{23}FN_4O_2S$: C, 64.92; H, 5.01; N, 12.11; Found: C, 64.95; H, 5.02; N, 12.13; MS m/z: $[M + H]^+$ 463.18.

4.2.2.13. 3-(1-(2-Fluoro-[1,1′-biphenyl]-4-yl)ethyl)-4-((furan-2-ylmethylene)amino)-1H-1,2,4-triazole-5(4H)-thione (6m). Dark brown powder; mp 197 °C. IR (KBr) cm^{-1}: 3141, 1569, 1221; ^1H NMR (DMSO-d_6, 400 MHz): δ 1.62 (d, J = 7.2 Hz, 3H, CH_3), 4.46 (q, J = 7.2 Hz, 1H, C*H*), 6.76 (dd, J = 3.6 Hz, 2.0 Hz, 1H, Ar*H*), 7.18 (dd, J = 8.0 Hz, 2.0 Hz, 1H, Ar*H*), 7.24 (dd, J = 12.0 Hz, 1.6 Hz, 1H, Ar*H*), 7.29 (dd, J = 3.6 Hz, 0.4 Hz, 1H, Ar*H*), 7.35−7.40 (m, 1H, Ar*H*), 7.42−7.50 (m, 5H, Ar*H*), 8.06 (d, J = 2.0 Hz, 1H, Ar*H*), 9.77 (s, 1H, N=C*H*), 13.98 (s, 1H, N*H*) ppm; ^{13}C NMR (DMSO-d_6, 101 MHz): δ 19.2, 35.3, 113.1, 115.5 (d, J = 24.2 Hz), 120.0, 124.0 (d, J = 3.0 Hz), 126.9 (d, J = 13.1 Hz), 127.9, 128.7, 128.8 (d, J = 3.0 Hz), 130.9 (d, J = 4.0 Hz), 134.9, 143.1 (d, J = 8.1 Hz), 147.4, 148.2, 151.5, 153.0, 159.0 (d, J = 247.5 Hz), 161.6 ppm; Anal. Calcd for $C_{21}H_{17}FN_4OS$: C, 64.27; H, 4.37; N, 14.28. Found: C, 64.30; H, 4.38; N, 14.33; MS m/z: $[M + H]^+$ 393.16.

4.3. X-ray Data Collection and Structure Determination. Crystal data for **6a**: $C_{23}H_{19}FN_4S$, M = 402.48, triclinic, a = 7.4736(4), b = 10.2302(5), c = 13.6434(7) Å, α = 100.3807(7), β = 100.3547(8), γ = 91.5795(8) °, U = 1007.40(9) $Å^3$, T = 150(2) K, and space group $P\bar{1}$. Data were collected on a Bruker APEX 2 CCDD diffractometer using graphite-monochromated Mo Kα radiation, λ = 0.71073 Å.[36] Z = 2, D_c = 1.327 g cm^{-3}, $F(000)$ = 420, yellow, dimensions 0.47 × 0.27 × 0.11 mm^3, μ = 0.19 mm^{-1}, 3.20 < 2θ < 61.2°, 16217 reflections measured, 6117 unique, and R_{int} = 0.018. The structure was solved by direct methods and refined by full-matrix least squares on F^2.[37,38] $wR2$ = 0.116 (all data, 277 parameters); $R1$ = 0.040 [5213 data with $F^2 > 2\sigma(F^2)$]. The F atom was modeled as two-fold disordered at atoms C(14) and C(16) with major occupancy {75.5(2)%}at C(14). CCDC 1511104 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

4.4. Analgesic Activity Methods. *4.4.1. Tail Flick Method.* The analgesic activity was determined by the tail immersion method.[30,31] The distal 5 cm portion of tails of swiss albino mice of either sex weighing between 20 and 40 g were marked and immersed into the water bath maintained at exactly 55 ± 1.0 °C. The reaction time for withdrawal of tails as a reflex action from the water was recorded with and without oral administration of the test compounds **6**(**a**−**m**) along with standard flurbiprofen (10 mg/kg). The readings for each test compound were recorded for six albino mice at the time intervals of 0.5, 1, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, and 4.5 h, which are tabulated in Table 2. All the results are presented as mean ± SEM, and one-way ANOVA considering P < 0.05 as significant is used to calculate the statistical significance.

4.4.2. Hot Plate Method. The central analgesic effect was measured by the hot plate method originally developed by MacDonald and co-workers.[39] Animals (20−40 g) were divided into groups of six, and each Swiss albino mouse was placed individually on the surface of the hot plate maintained at 52 ± 1 °C until it licked its forepaw or jumped, and the time was noted cautiously. The cut off time in the absence of response was set to 15 s to prevent tissue damage. The first group served as the positive control with no oral administration; the second group of animals were given a dose of the standard flurbiprofen (10 mg/kg) while other groups were administered with the test compounds **6**(**a**−**m**). Readings were recorded after each 30 min interval till 4.5 h and are given in Table 3. The analgesic activity for each mouse was calculated as a percentage of the maximum possible effect (MPE %), where MPE % = [{(test latency − control latency)/(cut-off point − control latency)} × 100].

Statistical Analysis: The results are presented as mean ± SEM, one-way ANOVA considering P < 0.05 as significant is used to calculate the statistical significance.

4.4.3. Acetic Acid Induced Writhing Method. Swiss albino mice divided into groups of six, weighing between 20 and 40 g, were used for the evaluation of analgesic activity by acetic acid induced writhing method.[34,35] The animals of group I, serving as control, were treated with 10 mL/kg of normal saline administered orally, group II was treated with 10 mg/kg of flurbiprofen as the standard drug, while the rest of the groups

https://doi.org/10.1021/acsomega.1c05222
ACS Omega 2021, 6, 31348−31357

were treated with 10 mg/kg of the test compounds. After 30 min, each mouse was administered with acetic acid (0.6% in normal saline) and was immediately shifted into a visible glass chamber for counting of induced writhes during the next 30 min. Percent analgesic activity of each group was determined as

$$\text{percent inhibition} = \{(X - Y)/X\} \times 100$$

where X = average count of writhes for the control group and Y = average count of writhes for the group administered with test compound.

The results of acetic acid induced writhing are presented in Table 4.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c05222.

Procedures for the synthesis of compound **2** and **3**, ^{1}H NMR, ^{13}C NMR, and mass spectra of the synthesized compounds (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Muhammad Zia-ur-Rehman − *Applied Chemistry Research Centre, PCSIR Laboratories Complex, Lahore 54600, Pakistan*; Email: rehman_pcsir@yahoo.com

Rubina Munir − *Department of Chemistry, Kinnaird College for Women, Lahore 54000, Pakistan*; orcid.org/0000-0001-8015-5974; Email: rubina.munir@kinnaird.edu.pk, organist94@gmail.com

Authors

Muhammad Zaheer − *Applied Chemistry Research Centre, PCSIR Laboratories Complex, Lahore 54600, Pakistan*

Nadia Jamil − *College of Earth & Environmental Sciences, University of the Punjab, Lahore 54590, Pakistan*

Saiqa Ishtiaq − *University College of Pharmacy, University of Punjab, Lahore 54000, Pakistan*

Rahman Shah Zaib Saleem − *Department of Chemistry and Chemical Engineering, Syed Babar Ali School of Science and Engineering, Lahore University of Management Sciences, Lahore 54792, Pakistan*

Mark R. J. Elsegood − *Chemistry Department, Loughborough University, Loughborough LE11 3TU, U.K.*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.1c05222

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors extended their gratitude to PCSIR Laboratories Complex, Lahore, and the University College of Pharmacy, University of Punjab, Lahore, for the provision of research facilities. No external funding was obtained for this research.

■ ABBREVIATIONS

NMR, nuclear magnetic resonance; NSAIDs, non-steroidal anti-inflammatory drugs; GI, gastrointestinal; COX, cyclo-oxygenase; DMSO, dimethyl sulfoxide; mp, melting point; FT-IR, Fourier transform infrared; TLC, thin-layer chromatography; MPE, maximum possible effect; CCDC, Cambridge Crystallographic Data Centre

■ REFERENCES

(1) Sahu, J. K.; Ganguly, S.; Kaushik, A. Triazoles: A valuable insight into recent developments and biological activities. *Chin. J. Nat. Med.* **2013**, *11*, 456−465.

(2) Duan, J.-R.; Liu, H.-B.; Jeyakkumar, P.; Gopala, L.; Li, S.; Geng, R.-X.; Zhou, C.-H. Design, synthesis and biological evaluation of novel Schiff base-bridged tetrahydroprotoberberine triazoles as a new type of potential antimicrobial agents. *MedChemComm* **2017**, *8*, 907−916.

(3) Lau, Y. H.; Rutledge, P. J.; Watkinson, M.; Todd, M. H. Chemical sensors that incorporate click-derived triazoles. *Chem. Soc. Rev.* **2011**, *40*, 2848−2866.

(4) Kumbhare, R. M.; Kosurkar, U. B.; Janaki Ramaiah, M.; Dadmal, T. L.; Pushpavalli, S. N. C. V. L.; Pal-Bhadra, M. Synthesis and biological evaluation of novel triazoles and isoxazoles linked 2-phenyl benzothiazole as potential anticancer agents. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 5424−5427.

(5) Shakeel-u-Rehman; Masood-ur-Rahman; Tripathi, V. K.; Singh, J.; Ara, T.; Koul, S.; Farooq, S.; Kaul, A. Synthesis and biological evaluation of novel isoxazoles and triazoles linked 6-hydroxycoumarin as potent cytotoxic agents. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 4243−4246.

(6) Evangelin, M. P.; Yanamadala Pavani, D.; Kumar, P.; Monica, K.; Venni, D.; Sukeerthika, S. U.; Anusha, A. R. An updated review on 1, 2, 4 Triazoles. *J. Pharmacogn. Phytochem.* **2019**, *8*, 292−299.

(7) Almasirad, A.; Shafiee, A.; Abdollahi, M.; Noeparast, A.; Shahrokhinejad, N.; Vousooghi, N.; Tabatabai, S. A.; Khorasani, R. Synthesis and analgesic activity of new 1, 3, 4-oxadiazoles and 1, 2, 4-triazoles. *Med. Chem. Res.* **2011**, *20*, 435−442.

(8) Ahirwar, J.; Ahirwar, D.; Lanjhiyana, S.; Jha, A. K.; Dewangan, D.; Badwaik, H. Analgesic and Anti-inflammatory Potential of Merged Pharmacophore Containing 1, 2, 4-triazoles and Substituted Benzyl Groups via Thio Linkage. *J. Heterocycl. Chem.* **2018**, *55*, 2130−2141.

(9) Srinivasa, S. B.; Poojary, B.; Brahmavara, U.; Das, A. J.; Middha, S. K. Anti-Inflammatory, Radical Scavenging Mechanism of New 4-Aryl-[1,3]-thiazol-2-yl-2-quinoline Carbohydrazides and Quinolinyl [1,3]-thiazolo [3,2-b][1,2,4] triazoles. *ChemistrySelect* **2018**, *3*, 12478−12485.

(10) Joshi, K. A.; Dhalani, J. M.; Bhatt, H. B.; Kapadiya, K. M. Synthesis of some new 6-aryl-3-(4-isopropylphenyl)[1, 2, 4] triazolo [3, 4-b][1, 3, 4] thiadiazoles and its anti-microbial study. *Curr. Chem. Lett.* **2021**, *10*, 479−488.

(11) Boraei, A. T. A.; Singh, P. K.; Sechi, M.; Satta, S. Discovery of novel functionalized 1, 2, 4-triazoles as PARP-1 inhibitors in breast cancer: Design, synthesis and antitumor activity evaluation. *Eur. J. Med. Chem.* **2019**, *182*, 111621.

(12) Khan, I.; Khan, A.; Ahsan Halim, S.; Saeed, A.; Mehsud, S.; Csuk, R.; Al-Harrasi, A.; Ibrar, A. Exploring biological efficacy of coumarin clubbed thiazolo [3, 2−b][1, 2, 4] triazoles as efficient inhibitors of urease: a biochemical and in silico approach. *Int. J. Biol. Macromol.* **2020**, *142*, 345−354.

(13) Klen, E. É.; Nikitina, I. L.; Makarova, N. N.; Miftakhova, A. F.; Ivanova, O. A.; Khaliullin, F. A.; Alekhin, E. K. 3-Substituted thietane-1, 1-dioxides: synthesis, antidepressant activity, and in silico prediction of their pharmacokinetic and toxicological properties. *Pharm. Chem. J.* **2017**, *50*, 642−648.

(14) Wan, K.; Zhou, C.-H. Synthesis of novel halobenzyloxy and alkoxy 1, 2, 4-triazoles and evaluation for their antifungal and antibacterial activities. *Bull. Korean Chem. Soc.* **2010**, *31*, 2003−2010.

(15) Abuelhassan, A. H.; Badran, M. M.; Hassan, H. A.; Abdelhamed, D.; Elnabtity, S.; Aly, O. M. Design, synthesis, anticonvulsant activity, and pharmacophore study of new 1, 5-diaryl-1 H-1, 2, 4-triazole-3-carboxamide derivatives. *Med. Chem. Res.* **2018**, *27*, 928−938.

(16) Tawfik, S. S.; Farahat, A. A.; A-A El-Sayed, M.; Tantawy, A. S.; Bagato, O.; Ali, M. A. Synthesis and Anti-influenza Activity of Novel

Thiadiazole, Oxadiazole and Triazole Based Scaffolds. *Lett. Drug Des. Discovery* **2018**, *15*, 363−374.
(17) Sehajpal, S.; Prasad, D. N.; Singh, R. K. Prodrugs of non-steroidal anti-inflammatory drugs (NSAIDs): a long march towards synthesis of safer NSAIDs. *Mini-Rev. Med. Chem.* **2018**, *18*, 1199−1219.
(18) Bua, S.; Di Cesare Mannelli, L.; Vullo, D.; Ghelardini, C.; Bartolucci, G.; Scozzafava, A.; Supuran, C. T.; Carta, F. Design and synthesis of novel nonsteroidal anti-inflammatory drugs and carbonic anhydrase inhibitors hybrids (NSAIDs−CAIs) for the treatment of rheumatoid arthritis. *J. Med. Chem.* **2017**, *60*, 1159−1170.
(19) Ammar, Y. A.; Salem, M. A.; Fayed, E. A.; Helal, M. H.; El-Gaby, M. S. A.; Thabet, H. K. Naproxen derivatives: synthesis, reactions, and biological applications. *Synth. Commun.* **2017**, *47*, 1341−1367.
(20) Kalgutkar, A. S.; Rowlinson, S. W.; Crews, B. C.; Marnett, L. J. Amide derivatives of meclofenamic acid as selective cyclooxygenase-2 inhibitors. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 521−524.
(21) Savjani, J. K.; Mulamkattil, S.; Variya, B.; Patel, S. Molecular docking, synthesis and biological screening of mefenamic acid derivatives as anti-inflammatory agents. *Eur. J. Pharmacol.* **2017**, *801*, 28−34.
(22) Begum, M. Y.; Abbulu, K.; Sudhakar, M. Design and evaluation of flurbiprofen liposomes. *J. Pharm. Res.* **2011**, *4*, 653−655.
(23) Wu, B.; Li, M.; Li, K.; Hong, W.; Lv, Q.; Li, Y.; Xie, S.; Han, J.; Tian, B. Cell penetrating peptide TAT-functionalized liposomes for efficient ophthalmic delivery of flurbiprofen: Penetration and its underlying mechanism, retention, anti-inflammation and biocompatibility. *Int. J. Pharm.* **2021**, *598*, 120405.
(24) Fukumoto, A.; Tajima, K.; Hori, M.; Toda, Y.; Kaku, S.; Matsumoto, H. Analgesic effect of S (+)-flurbiprofen plaster in a rat model of knee arthritis: analysis of gait and synovial fluid prostaglandin E2 levels. *J. Pharm. Pharmacol.* **2018**, *70*, 929−936.
(25) Kimmey, M. B. NSAID, ulcers, and prostaglandins. *J. Rheumatol., Suppl.* **1992**, *36*, 68−73.
(26) Lanza, F. L. A guideline for the treatment and prevention of NSAID-induced ulcers. *Am. J. Gastroenterol.* **1998**, *93*, 2037−2046.
(27) Zaheer, M.; Zia-ur-Rehman, M.; Jamil, N.; Arshad, M. N.; Siddiqui, S. Z.; Asiri, A. M. Efficient green synthesis of N′-benzylidene-2-(2-fluorobiphenyl) propanehydrazides: crystal structure and anti-oxidant potential. *J. Chem. Res.* **2015**, *39*, 668−673.
(28) Liu, W.; Yin, P.; Liu, X.; Chen, W.; Chen, H.; Liu, C.; Qu, R.; Xu, Q. Microwave assisted esterification of free fatty acid over a heterogeneous catalyst for biodiesel production. *Energy Convers. Manage.* **2013**, *76*, 1009−1014.
(29) El Ashry, E. S. H.; Ramadan, E. S.; Abdel Hamid, H.; Hagar, M. Microwave-Assisted Synthesis of Quinoline Derivatives from Isatin. *Synth. Commun.* **2005**, *35*, 2243−2250.
(30) Adeyemi, O. O.; Okpo, S. O.; Okpaka, O. The analgesic effect of the methanolic extract of Acanthus montanus. *J. Ethnopharmacol.* **2004**, *90*, 45−48.
(31) Vogel, H. G.; Vogel, W. H. Analgesic, anti-inflammatory, and antipyretic activity. *Drug Discovery and Evaluation*; Springer: Berlin, 1997; pp 360−420.
(32) Shanmugasundaram, P.; Venkataraman, S. Anti-nociceptive activity of Hygrophila auriculata (Schum) Heine. *Afr. J. Tradit., Complementary Altern. Med.* **2005**, *2*, 62−69.
(33) Verma, P. R.; Joharapurkar, A. A.; Chatpalliwar, V. A.; Asnani, A. J. Antinociceptive activity of alcoholic extract of Hemidesmus indicus R. Br. in mice. *J. Ethnopharmacol.* **2005**, *102*, 298−301.
(34) Hinschberger, A.; Butt, S.; Lelong, V.; Boulouard, M.; Dumuis, A.; Dauphin, F.; Bureau, R.; Pfeiffer, B.; Renard, P.; Rault, S. New benzo [h][1, 6] naphthyridine and azepino [3, 2-c] quinoline derivatives as selective antagonists of 5-HT4 receptors: binding profile and pharmacological characterization. *J. Med. Chem.* **2003**, *46*, 138−147.
(35) Nascimento, M. V. M.; Galdino, P. M.; Florentino, I. F.; Sampaio, B. L.; Vanderlinde, F. A.; de Paula, J. R.; Costa, E. A. Antinociceptive effect of Lafoensia pacari A. St.-Hil. independent of anti-inflammatory activity of ellagic acid. *J. Nat. Rem.* **2011**, *65*, 448−454.
(36) *APEX 2 and SAINT software for CCD diffractometers*; Bruker AXS Inc.: Madison, USA, 2012.
(37) Sheldrick, G. M. A short history ofSHELX. *Acta Crystallogr., Sect. A: Found. Crystallogr.* **2008**, *64*, 112−122.
(38) Sheldrick, G. M. Crystal structure refinement with SHELXL. *Acta Crystallogr., Sect. C: Struct. Chem.* **2015**, *71*, 3−8.
(39) MacDonald, A. D.; Woolfe, G.; Bergel, F.; Morrison, A. L.; Rinderknecht, H. Analgesic action of pethidine derivatives and related compounds. *Br. J. Pharmacol. Chemother.* **1946**, *1*, 4−14.

https://doi.org/10.1021/acsomega.1c05222
ACS Omega 2021, 6, 31348−31357