Received: 10 May 2017 | Revised: 7 June 2017 | Accepted: 15 July 2017

DOI: 10.1111/cbdd.13076

RESEARCH LETTER

WILEY

Screening of curcumin-derived isoxazole, pyrazoles, and pyrimidines for their anti-inflammatory, antinociceptive, and cyclooxygenase-2 inhibition

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Curcumin has shown pharmacological properties against different phenotypes of various disease models. Different synthetic routes have been employed to develop its numerous derivatives for diverse and improved therapeutic roles. In this study, we have synthesized curcumin derivatives containing isoxazole, pyrazoles, and pyrimidines and then the synthesized molecules were evaluated for their anti-inflammatory and antinociceptive activities in experimental animal models. Acute toxicity of synthesized molecules was evaluated in albino mice by oral administration. Any behavioral and neurological changes were observed at dose of 10 mg/kg body weight. Additionally, cyclooxygenase-2 (COX-2) enzyme inhibition studies were performed through in vitro assays. In vivo anti-inflammatory studies showed that curcumin with pyrimidines was the most potent anti-inflammatory agent which inhibited induced edema from 74.7% to 75.9%. Compounds **7**, **9**, and **12** exhibited relatively higher prevention of writhing episodes than any other compound with antinociceptive activity of 73.2%, 74.9%, and 71.8%, respectively. This was better than diclofenac sodium (reference drug, 67.1% inhibition). Similarly, COX-2 in vitro inhibition assays results revealed that compound **12** (75.3% inhibition) was the most potent compound. Molecular docking studies of **10**, **11**, and **12** compounds in human COX-2 binding site revealed the similar binding modes as that of other COX-2-selective inhibitors.

KEYWORDS
anti-inflammatory, COX-2 inhibition, curcumin derivatives, molecular modeling

Chemically curcumin is diferuloylmethane which has attracted much attention of medicinal chemists for various diseases and therapeutic agents development. It has shown its pharmacological safety and wide range of biological activities such as antibacterial to anticancer agent[1–4]. Currently, curcumin is acclaimed to be one of the most widely researched naturally occurring chemopreventive agent which is cytoprotective to healthy human cells[5–7].

In spite of important therapeutic application, limited therapeutic utility concerns are associated with curcumin because of its poor absorption and fast metabolism under physiological conditions[8]. Active methylene and keto moiety are believed to be responsible for its rapid metabolism. In order to circumvent the problem of rapid metabolism and to improve its pharmacokinetics profile, several synthetic modifications have been studied on carbonyl and active methylene moiety[9].

In present study, isoxazole, N-substituted pyrazoles, and pyrimidine ring were incorporated in this focused segment of curcumin. Nitrogen heterocyclic moieties such as pyrazoles and pyrimidines containing derivatives gained considerable attention in medicinal chemistry for

their broad-spectrum pharmacological activities such as antimicrobial, anti-inflammatory, analgesic, enzyme inhibition, antioxidant, and anticancer. These moieties have leading position in drug designing as important pharmacophore[10–15].

Inflammation is the body response to injury of cells and tissues due to different factors such as infections, chemicals, thermal, and mechanical injuries. Bradykinins and cytokines (pro-inflammatory mediators) mediate inflammation that increases the prostaglandins synthesis rate in the body. Inhibition of prostaglandins by non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin, diclofenac sodium, indomethacin and cyclooxygenase inhibitors (COXIBs) such as celecoxib, rofecoxib, and valdecoxib is in turn mediated by selective inhibition of the cyclooxygenases (COXs) particularly COX-2[11,16,17].

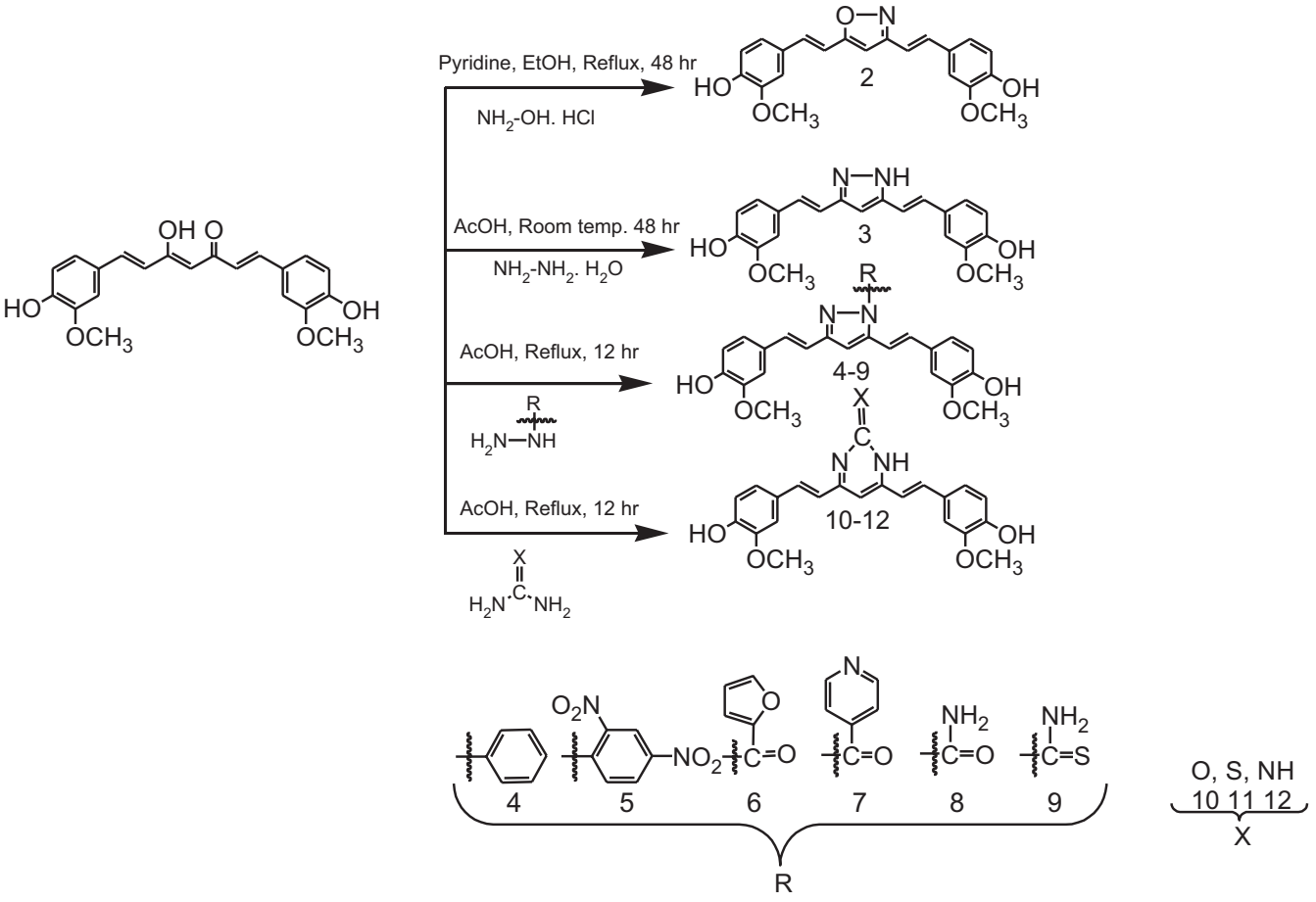
COXs are present in two major isoforms such as cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2). COX-1 is constitutive enzyme that produced physiological prostaglandins (PGs), whereas pathological PGs are produced by the inducible isoform COX-2. COX-2 is hyperexpressed at sites of inflammation, infection, and cancer. COX-2 overexpression leads to elevated production of prostanoids (a subclass of PGs) which are important for driving disease pathogenesis[18–20].

Gastrointestinal bleeding, irritation, and gastric ulcer are associated with these classical NSAIDs and non-selective

TABLE 1 Physiochemical and cLogP of curcumin–isoxazoles, pyrazoles/pyrimidines

Compound	M.P. [a] (°C)	Yield (%)	R_f [b]	cLogP
2	166–168	51.6	0.82	3.87
3	202–204	39.2	0.76	3.99
4	126–128	65.3	0.81	5.94
5	114–116	87.5	0.84	5.68
6	136–138	67.2	0.78	5.92
7	112–114	77.1	0.81	5.23
8	206–208	90.1	0.78	3.50
9	102–104	90.1	0.82	4.72
10	105–107	60.6	0.81	2.13
11	102–104	75.1	0.84	2.64
12	98–100	70.0	0.81	2.43

[a]Melting points were uncorrected.
[b]R_f values were measured in DCM/MeOH, 25:1.



SCHEME 1 Protocol for synthesis of curcumin isoxazole and pyrazoles/pyrimidines

COXIBs; however, COX-2-selective inhibitors such as rofecoxib and valdecoxib found to possess severe cardiovascular effects, so these have been withdrawn from the market[21–23].

In this situation, we wanted to synthesize and biologically assay the natural compound (curcumin) derivatives that can lead the medicinal chemist community to develop therapeutic intervention for various inflammations caused by COX-2 enzyme. In the best of our knowledge, curcumin–isoxazole, pyrazoles, and pyrimidines had never been studied for anti-inflammatory, antinociceptive, and COX-2 inhibition.

Isoxazole (2), N-substituted pyrazoles (3–9), and pyrimidine ring (10–12) containing curcumin derivatives were synthesized by interaction of curcumin with hydroxyl amine hydrochloride, hydrazine hydrate, phenyl hydrazine, 2,4-dinitrophenyl hydrazine, furoic hydrazide, isoniazid, semicarbazide, thiosemicarbazide, urea, thiourea, and guanidine, respectively. Synthetic route and structure of these curcumin derivatives are presented in Scheme 1, while the physiochemical data of synthesized compounds are shown in Table 1 (analytical data, see supplementary information, Table S1). All the synthesized compounds were confirmed by performing IR, ^{1}HNMR, and ^{13}CNMR spectral studies (Supporting information).

The anti-inflammatory activity of synthesized compounds was evaluated by carrageenan-induced paw edema in experimental animal models which is globally recognized and is believed to be biphasic. Early phase (1–2 hr) is predominantly a non-phagocytic edema followed by a late phase (up to 4 hr) with increased edema formation. Early phase of this model is attributed to release of bradykinin, histamine, serotonin, and prostaglandins synthesis in surroundings of damaged tissue.

TABLE 2 Anti-inflammatory and antinociceptive activity of curcumin–isoxazole, pyrazoles/pyrimidines

	Anti-inflammatory activity				**Antinociceptive activity**	
	Paw edema in mm (mean ± *SEM*), *n* = 6, (% inhibition)					
Compound	**0 hr**	**1 hr**	**2 hr**	**3 hr**	**Total writhing count (mean ± *SEM*), *n* = 6**	**% inhibition**
2	1.93 ± 0.04 (−)	2.89 ± 0.12 (7.7)	2.83 ± 0.15 (35.7)	2.80 ± 0.11 (66.1)[c]	22.4 ± 1.1	61.0
3	1.95 ± 0.05 (−)	2.91 ± 0.11 (7.7)	2.87 ± 0.16 (34.3)	2.80 ± 0.17 (66.9)[d]	23.3 ± 2.4	59.5
4	1.94 ± 0.08 (−)	2.88 ± 0.14 (9.6)	2.78 ± 0.13 (40.0)	2.73 ± 0.14 (69.3)[e]	41.1 ± 2.1	28.5
5	1.94 ± 0.08 (−)	2.87 ± 0.12 (10.6)	2.81 ± 0.11 (37.8)	2.76 ± 0.12 (68.1)[f]	22.6 ± 2.4	60.7
6	1.93 ± 0.05 (−)	2.85 ± 0.11 (11.5)	2.78 ± 0.14 (39.3)	2.73 ± 0.11 (68.9)[g]	19.4 ± 2.2	66.3
7	1.95 ± 0.06 (−)	2.80 ± 0.13 (18.3)	2.63 ± 0.09 (51.4)	2.61 ± 0.11 (74.3)[h]	15.4 ± 2.2	73.2
8	1.98 ± 0.01 (−)	2.89 ± 0.15 (12.5)	2.81 ± 0.13 (40.7)	2.78 ± 0.11 (68.9)[i,g]	42.4 ± 1.6	26.3
9	1.96 ± 0.04 (−)	2.87 ± 0.09 (11.5)	2.68 ± 0.16 (48.6)	2.63 ± 0.08 (73.9)[j]	14.4 ± 3.0	74.9
10	1.95 ± 0.05 (−)	2.77 ± 0.12 (21.2)	2.69 ± 0.12 (47.1)	2.60 ± 0.09 (74.7)[k]	19.2 ± 1.2	66.6
11	1.95 ± 0.05 (−)	2.91 ± 0.07 (7.7)	2.89 ± 0.11 (32.8)	2.60 ± 0.09 (74.7)[l,k]	32.4 ± 1.6	43.6
12	1.97 ± 0.04 (−)	2.77 ± 0.11 (19.2)	2.72 ± 0.08 (43.6)	2.59 ± 0.12 (75.9)[m]	16.2 ± 2.5	71.8
Curcumin	1.99 ± 0.04 (−)	2.80 ± 0.13 (22.1)	2.75 ± 0.04 (45.7)	2.72 ± 0.14 (71.6)[n]	24.1 ± 2.2	58.1
Standard drug [a,b]	1.94 ± 0.05 (−)	2.85 ± 0.14 (12.5)	2.63 ± 0.12 (50.7)	2.34 ± 0.12 (84.4)[o]	18.9 ± 1.9	67.1
Control[c]	1.95 ± 0.04 (−)	2.99 ± 0.11 (−)	3.35 ± 0.12 (−)	4.52 ± 0.11 (−)	57.5 ± 2.2	–

[a]Indomethacin for anti-inflammatory assay.

[b]Diclofenac sodium for antinociceptive assay.

[c]0.9% saline, significantly different values are represented by different letters (c–o) using one way ANOVA at $p < .05$.

TABLE 3 % inhibition of COX-2 by curcumin- isoxazole, pyrazoles, and pyrimidines and ulcer index

Compound	% inhibition of COX-2 at 10 μM	Ulcer index
2	49.3	6.0
3	47.7	6.0
4	32.5	15.0
5	31.0	14.0
6	32.2	10.0
7	55.4	7.0
8	32.5	23.0
9	33.8	13.0
10	66.2	1.0
11	67.7	1.0
12	75.3	1.0
Curcumin	19.1	–
Celecoxib	76.5	–
Diclofenac sodium	–	5.0

The late phase is sustained by prostaglandins release and mediated by leukotrienes, bradykinin, and polymorphonuclear cells[23–25]. The anti-inflammatory data (Table 2) indicate that carrageenan-induced edema is significantly inhibited by tested compounds in late phase. Among the screened compounds (**2–12**), compounds **7**, **9** with pyridyl ring and carbazide moiety exhibited better anti-inflammatory activity by inhibition of 74.3% and 73.9% of induced edema. Moreover, anti-inflammatory activity (74.7%–75.9% inhibition) of curcumin derivatives with pyrimidines is better than curcumin–pyrazoles. Hence, we presume that the similar in vivo antiedematogenic effects of curcumin and its synthesized derivatives might be due to their poor bioavailability to induced edema tissues.

After evaluating the anti-inflammatory activity, ulcerogenic potential of all synthesized compounds and diclofenac sodium (reference drug) was evaluated after 4 hr intraperitoneal (10 mg/kg) administration[26]. Animals were killed by diethyl ether, and the stomachs were removed, opened along the greater curvature, washed, and cleaned with saline and examined for ulceration. Examination of mucosal layer was done using magnifying lens (Olympus BX 41TF, Tokyo, Japan) to detect macroscopically visible lesions. The compounds **10**, **11**, and **12** exhibited excellent safe profile (Table 3) compared to that of diclofenac sodium (UI = 5.0). These findings are supported with histological examination (Figure 1) of gastric mucosa. Similarly, among the series, compounds **2** and **3** also showed better ulcerogenic activity as compared to diclofenac sodium.

Acetic acid-induced writhing responses were established to evaluate the antinociceptive activity of curcumin derivatives. Injection of acetic acid attributed to significant release of endogenous mediators such as prostaglandins, bradykinins, pro-inflammatory cytokines, and substance P. Local peritoneal receptor could be attributed to abdominal writhing (pain) which is symbolized by abdominal muscle contraction accompanied by extension of forelimbs and body elongation[27,28]. Among the tested compounds, compounds **7**, **9**, and **12** have more prevention of writhing episodes (Table 2) than any other compound and showed the antinociceptive activity up to 73.2, 74.9 and 71.8, respectively, % which is better than diclofenac sodium (67.1% inhibition). Thus, it could be assumed that synthetic curcumin derivatives interfered the release of peripherally acting endogenous substances responsible for pain sensation. Moreover, the mode of action

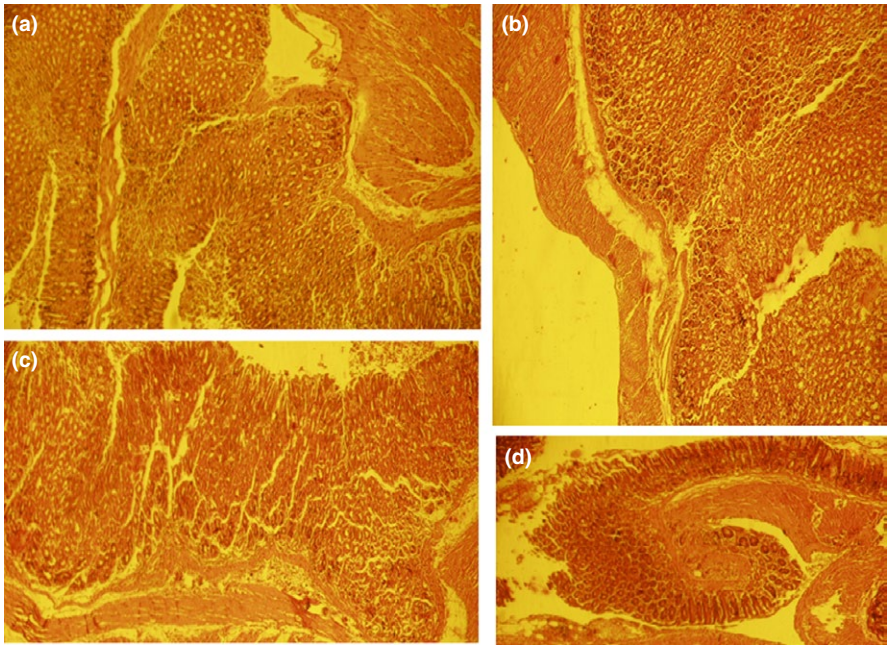


FIGURE 1 Histological examination of the stomach lining treated with (a) compound **10**, (b) compound **11**, (c) compound **12,** and (d) diclofenac sodium

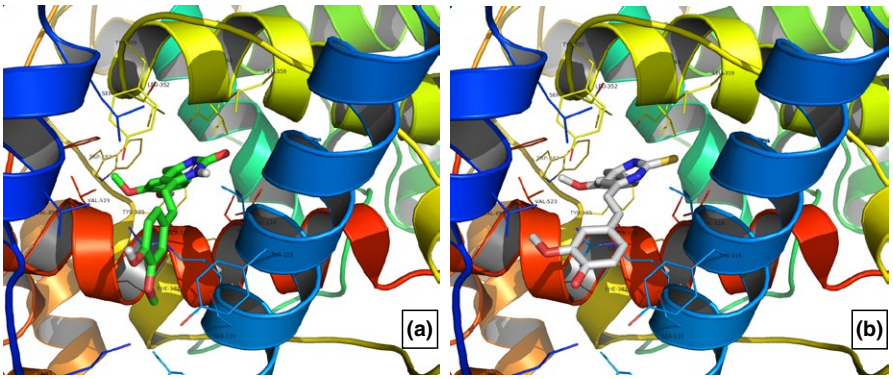


FIGURE 2 Molecular docking predicted binding modes of compound-**10** (green) and compound-**11** (white) in COX2 active site

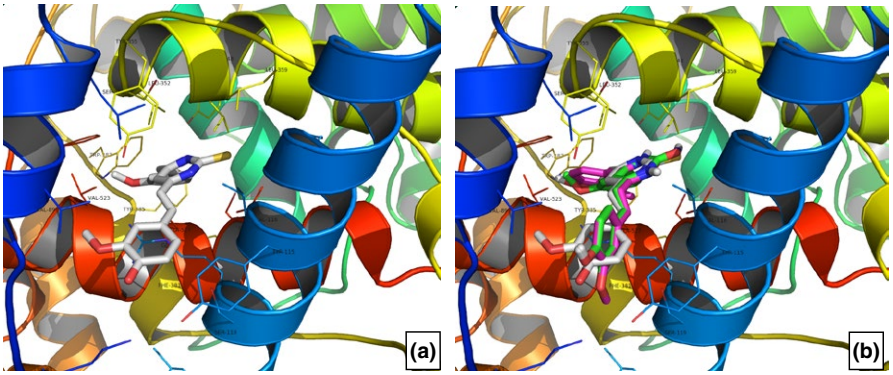


FIGURE 3 Molecular docking predicted binding modes of compound-**12** (magenta) in COX2 active site and superposition of top scored binding pose of each of best three most active compounds

of synthetic curcumin derivatives is similar to NSAID and analgesic such as diclofenac sodium.

All the compounds were evaluated for their inhibitory activity against human COX-2 enzyme employing screening assay kit (Item No. 760151, Cayman Chemical Company, USA). The % inhibition values calculated in triplicate and average inhibitory value of each compound is presented in Table 3. Standard selective COX-2 inhibitor, celecoxib was used as reference drug. Results demonstrated that compound **12** exhibited 75.3% inhibition that is comparable with reference drug (76.5% inhibition). Again curcumin with pyrimidines is better than curcumin–pyrazoles with relatively higher inhibitory activities.

Dose–response curves were drawn using GRAPHPAD PRISM 7.0 (GraphPad Software, Inc. USA) to compare the inhibition effects of compounds **10**, **11**, and **12** with curcumin. The results revealed that the IC_{50} of compounds **10** (7.6 μM), **11** (7.6 μM), and **12** (6.8 μM) was 4.0- to 4.5-fold less than curcumin (30.5 μM) (see supporting information, Figure. S1). More synthetic chemistry optimization of curcumin derivatives will lead us to develop clear picture of structure activity relationship. Further to investigate the plausible binding modes of the synthesized compounds against COX-2 enzyme, molecular docking simulation studies of the compounds **10**, **11**, and **12** were performed using the Glide docking software. Before docking studies, different X-rays resolved structures of COX-2 enzyme from protein data bank (www.rcsb.org) were investigated. The human-isolated enzyme structure (5f1a)[29] with the resolution 2.38 Å with R free value of 0.218 was selected for docking studies. Upon docking simulations, three most active compounds yielded similar as that of the selective COX-2 inhibitor SC-558. It might be due to their identical structural geometry than other compounds. The best docking pose of compound **10** (Figure 2a), 11 (Figure 2b), and 12 (Figure 3a) yielded −7.59, −6.97, and −6.74 Kcal/mol glide scores, respectively. All these compounds docked well in the binding site of the enzyme consisting of Tyr115, Val116, Tyr355, Met113, Val349, Tyr348, Tyr385, Try387, Met522, Leu352, Ala527, Val89, Tyr115, Lys83, Gln635 residues. On super positioning (Figure 3b) of best binding pose of each compound revealed that one phenyl ring of curcumin containing hydroxyl and methoxy groups buried deep inside the hydrophobic binding pocket, whereas other is exposed

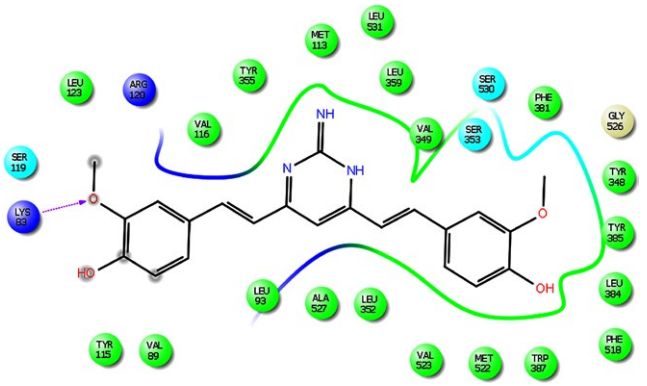


FIGURE 4 2D binding mode of most active compound, for clarity only interacting residues are displayed

toward the solvent side which might be due to the symmetrical nature of these compound. Further investigations revealed the hydrogen bond formation between N-H of Lys-83 and CH_3-O group of compound **12** (Figure 4). This interaction can also be observed in compound **11**, if the binding pose generates the similar conformation for phenyl ring.

In summary, curcumin-derived isoxazole, pyrazoles, and pyrimidines were assayed for anti-inflammatory, antinociceptive activities in experimental animal models, as well as in vitro COX-2 inhibition. Compound **9** exhibited highest antinociceptive activities (74.9%) compared to standard drugs diclofenac sodium. Similarly in vitro assays showed high percentage inhibition (75.3%) of the compound **12** against COX-2 enzyme. Docking experiments predicted the similar binding modes of the best active compounds in the active site of enzyme with reasonably good binding energies.

ACKNOWLEDGMENTS

We are thankful to Higher Education Commission (HEC) Pakistan for providing financial support of this project. We also thank Schrodinger Software Company for providing us the evaluation version of Schrodinger software suite.

CONFLICT OF INTEREST

Authors have no conflicts of interest.

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REFERENCES

[1] K. Liu, J. Chen, J. Chojnacki, S. Zhang, *Tetrahedron Lett.* **2013**, *54*, 2070.
[2] P. K. Sahu, P. K. Sahu, P. L. Sahu, D. D. Agarwal, *Bioorg. Med. Chem. Lett.* **2016**, *26*, 1342.
[3] X. Wei, Z.-Y. Du, X. Zheng, X.-X. Cui, A. H. Conney, K. Zhang, *Eur. J. Med. Chem.* **2012**, *53*, 235.
[4] N. S. Jha, S. Mishra, S. K. Jha, A. Surolia, *Electrochim. Acta* **2015**, *151*, 574.
[5] S. Raghavan, P. Manogaran, K. K. G. Narasimha, B. K. Kuppusami, P. Mariyappan, A. Gopalakrishnan, G. Venkatraman, *Bioorg. Med. Chem. Lett.* **2015**, *25*, 3601.
[6] M. Pröhl, U. S. Schubert, W. Weigand, M. Gottschaldt, *Coord. Chem. Rev.* **2016**, *307*, 32.
[7] S. M. Bayomi, H. A. El-Kashef, M. B. El-Ashmawy, M. N. Nasr, M. A. El-Sherbeny, N. I. Abdel-Aziz, A.-A. Magda, G. M. Suddek, S. M. El-Messery, M. A. Ghaly, *Eur. J. Med. Chem.* **2015**, *101*, 584.
[8] J. Lal, S. K. Gupta, D. Thavaselvam, D. D. Agarwal, *Chin. Chem. Lett.* **1067**, *2016*, 27.
[9] X. Fang, L. Fang, S. Gou, L. Cheng, *Bioorg. Med. Chem. Lett.* **2013**, *23*, 1297.
[10] A. Abbas, S. Murtaza, M. N. Tahir, S. Shamim, M. Sirajuddin, U. A. Rana, K. Naseem, H. Rafique, *J. Mol. Struct.* **2016**, *1117*, 269.
[11] S. D. Firke, S. B. Bari, *Bioorg. Med. Chem.* **2015**, *23*, 5273.
[12] S. Mutahir, J. Jończyk, M. Bajda, I. U. Khan, M. A. Khan, N. Ullah, M. Ashraf, S. Riaz, S. Hussain, M. Yar, *Bioorg. Chem.* **2016**, *64*, 13.
[13] N. U. H. Khan, S. Zaib, K. Sultana, I. Khan, B. Mougang-Soume, H. Nadeem, M. Hassan, J. Iqbal, *RSC Adv.* **2015**, *5*, 30125.
[14] V. G. Reddy, T. S. Reddy, V. L. Nayak, B. Prasad, A. P. Reddy, A. Ravikumar, S. Taj, A. Kamal, *Eur. J. Med. Chem.* **2016**, *122*, 164.
[15] M. F. Khan, M. M. Alam, G. Verma, W. Akhtar, M. Akhter, M. Shaquiquzzaman, *Eur. J. Med. Chem.* **2016**, *120*, 170.
[16] A.-M. Alaa, A. S. El-Azab, L. A. Abou-Zeid, K. E. H. ElTahir, N. I. Abdel-Aziz, R. R. Ayyad, A. M. Al-Obaid, *Eur. J. Med. Chem.* **2016**, *115*, 121.
[17] E. Iwalewa, O. Oyedapo, C. Adewunmi, V. Makunja, *J. Biol. Sci.* **2008**, *8*, 131.
[18] N. Handler, W. Jaeger, H. Puschacher, K. Leisser, T. Erker, *Chem. Pharm. Bull.* **2007**, *55*, 64.
[19] B. P. Bandgar, S. N. Kinkar, H. V. Chavan, S. S. Jalde, R. U. Shaikh, R. N. Gacche, *J. Enzyme Inhib. Med. Chem.* **2014**, *29*, 7.
[20] P. Świątek, M. Strzelecka, R. Urniaz, K. Gębczak, T. Gębarowski, K. Gąsiorowski, W. Malinka, *Bioorg. Med. Chem.* **2017**, *25*, 316.
[21] P. Singh, S. Kaur, J. Kaur, G. Singh, R. Bhatti, *J. Med. Chem.* **2016**, *59*, 3920.
[22] S. S. Undare, N. J. Valekar, A. A. Patravale, D. K. Jamale, S. S. Vibhute, L. S. Walekar, G. B. Kolekar, M. Deshmukh, P. V. Anbhule, *Bioorg. Med. Chem. Lett.* **2016**, *26*, 814.
[23] M. A. Abdel-Sayed, S. M. Bayomi, M. A. El-Sherbeny, N. I. Abdel-Aziz, K. E. H. ElTahir, G. S. Shehatou, A.-M. Alaa, *Bioorg. Med. Chem.* **2016**, *24*, 2032.
[24] E. Zangrando, M. Islam, M. A.-A. A. Islam, M. Sheikh, M. Tarafder, R. Miyatake, R. Zahan, M. Hossain, *Inorg. Chim. Acta* **2015**, *427*, 278.
[25] A. Rauf, G. Uddin, B. S. Siddiqui, A. Khan, H. Khan, M. Arfan, N. Muhammad, A. Wadood, *Phytomedicine* **2014**, *21*, 1509.
[26] H. S. Abd-Ellah, M. Abdel-Aziz, M. E. Shoman, E. A. Beshr, T. S. Kaoud, A.-S. F. Ahmed, *Bioorg. Chem.* **2016**, *69*, 48.
[27] F. Hasnain, K. H. Janbaz, M. A. Qureshi, *Pak. J. Pharm. Sci.* **2012**, *25*, 599.
[28] G. Uddin, A. Rauf, B. S. Siddiqui, N. Muhammad, A. Khan, S. U. A. Shah, *Phytomedicine* **2014**, *21*, 954.
[29] M. J. Lucido, B. J. Orlando, A. J. Vecchio, M. G. Malkowski, *Biochemistry* **2016**, *55*, 1226.

SUPPORTING INFORMATION

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How to cite this article: Ahmed M, Qadir MA, Hameed A, Imran M, Muddassar M. Screening of curcumin-derived isoxazole, pyrazoles, and pyrimidines for their anti-inflammatory, antinociceptive, and cyclooxygenase-2 inhibition. *Chem Biol Drug Des*. 2017;00:1–6. https://doi.org/10.1111/cbdd.13076