European Journal of Medicinal Chemistry 58 (2012) 478–484

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Short communication

Synthesis pharmacological evaluation and docking studies of pyrimidine derivatives

D. Giles [a,*], Karki Roopa [a], F.R. Sheeba [b], P.M. Gurubasavarajaswamy [a], Goli Divakar [a], Thomas Vidhya [a]

[a] Acharya & B.M. Reddy College of Pharmacy, Bangalore, Karnataka, India
[b] Mallige College of Pharmacy, Bangalore, Karnataka, India

ARTICLE INFO

Article history:
Received 2 July 2012
Received in revised form
27 September 2012
Accepted 28 September 2012
Available online 16 October 2012

Keywords:
Analgesic activity
Anti-Inflammatory activity
Antimicrobial activity
Cyclooxygenase
Indane-1,3-dione
Pyrimidine
Ulcerogenic activity

ABSTRACT

A new group of pyrimidine derivatives of indane-1,3-dione were synthesized aiming at the synthesis of new compounds acting as analgesic, anti-inflammatory and antimicrobial activity in a single component. The title compounds (**3a-l**) were synthesized from chalcone derivatives of indane-1,3-dione (**2a-l**) through cyclization reaction with urea. The synthesized compounds were characterized by FT-IR, ^{1}H NMR, mass spectral data, elemental analysis and evaluated for anti-inflammatory, analgesic, antibacterial and antifungal activities. The most active compound **3e**, was evaluated for its ulcerogenicity. Good anti-inflammatory property was observed for chlorophenyl substituted pyrimidine derivatives. It mainly binds with Pro 218 of **1CX2**, and the ligand could have caused much conformational changes in the protein structure than other derivatives. It also exhibits good analgesic and antimicrobial agent in a single component.

1. Introduction

Inflammation is a complex defensive mechanism of the body to any noxious stimulus [1]. Non-steroidal anti-inflammatory drugs are of huge benefit in the treatment of inflammatory disease [2,3]. The most common side effect associated with all currently available non-steroidal anti-inflammatory drugs are gastrointestinal hemorrhagica and ulceration [4–6]. In fact, while a number of mechanisms have been proposed to explain the pharmacological effects of non-steroidal anti-inflammatory drugs, it is believed that the inhibition of cyclooxygenase (COX) is one of the greatest importance. The discovery of isoform of the COX-1 and COX-2 enzymes opens a new perspective of COX-2 inhibitors used in therapeutics [7–9]. Consequently, a new generation of COX-2 selective inhibitors has been clinically used with the hope that they would exhibit a reduced risk in gastrointestinal events [10].

Pyrimidine ring system has attracted significant interest in medicinal chemistry over the past few decades. Scaffolds containing the pyrimidine heterocyclic have demonstrated a wide range of biological activity viz. anti-inflammatory [11–14], analgesic [11–13,15,16], antipyretic [16], antimicrobial [14,17–20] and anticancer [21–25] activities. Indane-1,3-dione exhibits wide range of biological activities covering anti-inflammatory [26–28], analgesic [26,29], anticoagulant [26,30], antimicrobial [26,31] and anticancer [26,32,33] activities.

Co-administration of multiple drugs for treatment of pain, inflammatory conditions associated with microbial infection is a major risk factor for the patients with impaired liver or kidney functions. A mono therapy of a drug with analgesic, anti-inflammatory and antimicrobial activity would be preferred form of the pharmacoeconomic and patient compliance point of view. This premise was one of the goals of our research aimed at the discovery of new pyrimidine derivatives that would possess analgesic, anti-inflammatory and antimicrobial agent in a single component. Combination of two or more biological active moieties increases or decreases the biological activity. Being involved in a research program aiming at finding out new structural leads that would act as potent anti-inflammatory, analgesic and antimicrobial agents, we have reported the synthesis, anti-inflammatory, analgesic and antimicrobial activities of some lead compounds comprising mainly pyrimidine derivatives of indane-1,3-dione.

* Corresponding author. Department of Pharmaceutical Chemistry, Acharya & B.M. Reddy College of Pharmacy, Soladevanahalli, Chikkabanavara Post, Bangalore 560 090, Karnataka, India. Tel.: +91 94487 36917.
E-mail address: sahayagiles@yahoo.com (D. Giles).

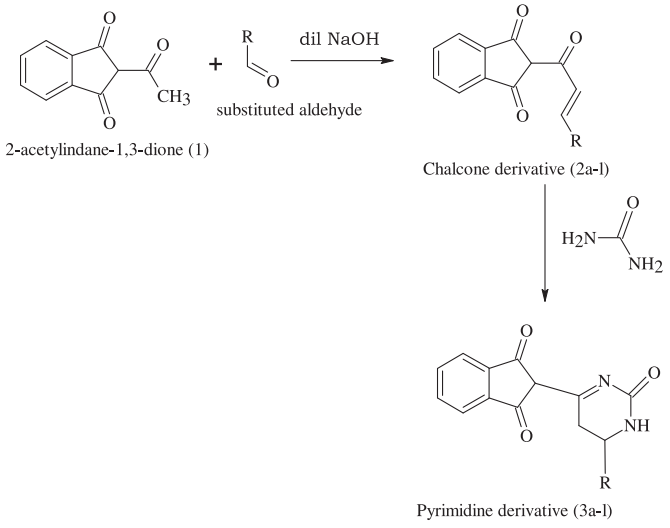
0223-5234/$ – see front matter © 2012 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.ejmech.2012.09.050

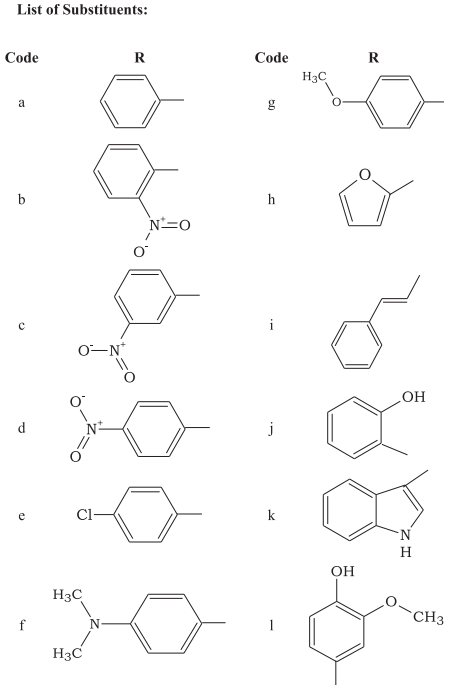
2. Results and discussion

2.1. Chemistry

Synthesis of the target compounds were performed according to the reactions illustrated in Scheme 1. 2-Acetylindane-1,3-dione (**1**) was treated with different aldehydes (**a-l**) to form chalcones of indane-1,3-dione (**2a-l**). The chalcones (**2a-l**) were further treated with urea to form pyrimidine derivatives of indane-1,3-dione (**3a-l**).

The desired pyrimidine analogs (**3a-l**) were confirmed by the spectral data. The IR spectra of products exhibited absorption bands at 3226–3394 cm^{-1}, assigned to NH group stretching vibration which was not observed for chalcone derivatives (**2a-l**). The stretching frequency at 1671–1709 cm^{-1} was assigned to C=O vibrations and the characteristic absorptions for C=N was also present. The main characteristic of the ^1H NMR for the pyrimidine series was the presence of a three doublet of doublet attributed by CH and CH_2 protons which was not observed in the chalcone derivatives. The CH proton adjacent to carbonyl carbon of indane-1,3-dione appeared as a singlet. All these results established the formation of target compounds. It was further confirmed by mass spectral data and elemental analysis.





Scheme 1. General scheme for the synthesis of pyrimidine derivatives of indane-1,3-dione.

2.2. Molecular modeling studies

2.2.1. Docking studies

Molecular docking studies of compounds **3a-l** were carried out using Molegro Virtual Docker 2010 in order to rationalize biological activity results. Docking also helps in understanding the various interactions between ligand and enzyme in the active site in detail. The crystal structure of COX-2 (PDB: **1CX2**) and COX-1 (PDB: **2OYE**) were used for docking study.

As per the docking study with **1CX2** the following results can be drawn. The synthesized series **3a-l** had MolDock score ranging from −130.970 to −150.445. Five hydrogen bonds were present in the derivative **3j**, which was the highest among the series. The carbonyl group of indane-1,3-dione mainly binds with NH_2 of Arg 44 in compounds **3b**, **3d**, **3g**, **3h**, **3i**, **3j** and **3k** which had hydrogen bond of 3.032, 3.038, 3.083, 2.824, 3.033, 3.105 and 2.538 Å respectively. Chloro group in phenyl ring binds with NH_2 of Pro 218 which was not observed in any other derivatives. Pyrimidine ring in this series binds mainly with OH of Ser 439, C=O of Lys 468, NH_2 of Arg 150, OH of Ser 471, NH_2 of Lys 468, C=O of Glu 465, C=O of Lys 465, NH_2 of Asn 43, NH of Gln 461, NH_2 of Arg 44. It was a clear indication that the pyrimidine ring had an enough role in binding of the drug with the COX-2. Oxygen of NO_2 binds with NH of Arg 44 in **3c** derivative with a bond length of 3.434 Å. Oxygen in furan ring of **3h** binds with NH_2 of Asn 43 with a bond length of 3.158 Å. In **3j** the OH in phenyl ring binds with C=O of Cys 141 and Gly 145 with bond length of 2.639 Å and 3.187 Å respectively. NH in indole ring of **3k** binds with C=O of Arg 469. OH and OCH_3 in **3l** binds with Cys 36 and Gln 461 with bond length of 3.552 Å and 3.520 Å respectively. This indicates the effect of substituent in phenyl ring.

The synthesized series **3a-l** had MolDock score ranging from −110.682 to −155.055 for **2OYE**. Seven hydrogen bonds were present in the derivative **3j**, which was the highest among the series. The MolDock score for the compound was −110.682. The carbonyl group of indane-1,3-dione mainly binds with NH_2 of Gln 44 in compounds **3a**, **3b**, **3d**, **3f**, **3g**, **3h**, **3i**, **3j** and **3l** which had good hydrogen bonding with the target. All the compounds except **3k** were binding with indane-1,3-dione. Pyrimidine ring in this series binds mainly with NH of His 43, NH of Gln 42, NH of Lys 468, OH of Tyr 64, OH of Tyr 130, NH of Gly 471, NH of Gln 44, OH of Thr 129. This clearly indicates the effect of indane-1,3-dione and pyrimidine ring in the ring system. The substituent's also binds with the target indicating the effect of substituent. Compound **3e** has a MolDock Score of −127.314 with three hydrogen bonds.

2.3. Biological evaluation

2.3.1. Anti-inflammatory activity

All the synthesized compounds were screened for their anti-inflammatory activity by carrageenan induced rat paw edema method. The results of anti-inflammatory activity indicated that compounds in pyrimidine series except **3g** and **3k** exhibited significant activity than that of control. None of the compounds in this series were much effective as standard. Steady increase in paw edema inhibition was observed for **3e** than other synthesized derivatives. Compound **3c** showed significant anti-inflammatory activity at 1 and 2 h of drug administration but the effect started decreasing from 3 h. The anti-inflammatory effect produced by **3c** at 1 h was higher than standard. Whereas, other compounds

showed moderate anti-inflammatory activity compared to that of standard drug indomethacin. Edema inhibition of **3e** at **3h** was found to be similar to that of standard at 4 h of drug administration. All these results indicate that pyrimidine series of indane-1,3-dione possess moderate anti-inflammatory activity.

The molecular modeling studies correlates well with pharmacological activity. In this regard, the compound **3e** showed selective inhibition of COX-2 than COX-1. Docking results were in concordance with the anti-inflammatory activity. Molecules binding with Arg 44 possess significant anti-inflammatory activity which could be possible because of the presence of both indane-1,-3-dione and pyrimidine ring. At the same time, binding of **3e** with Pro 218 in **1CX2** could have caused much conformational changes in the protein structure than other derivatives. This could be the reason for its good anti-inflammatory activity.

2.3.2. Ulcerogenic effect

The compounds **3e** showed good anti-inflammatory. So it was screened for ulcerogenicity. The tested **3e** showed significant reduction in ulcerogenic activity when compared to that of standard. This indicates that compound **3e** does not induce ulcer. Docking results also confirms the less affinity towards COX-1.

2.3.3. Analgesic activity

All the compounds were screened for analgesic activity by acetic acid induced writhing method in mice. The results indicate that some of the compounds in the series **3a**-**l** possess good activity. The percentage activity in this series ranges from 16% to 52%. Compound **3f** showed excellent analgesic activity whereas **3a**, **3b**, **3c**, **3d**, **3e**, **3g**, **3j**, **3k** and **3l** showed moderate to good analgesic activity. The activity of these compounds ranges from 21% to 44%. Least active analgesic in this series was **3i**. Presence of dimethyl amino group as substituent emerged as a most active analgesic agent among the synthesized series. Moderate analgesic activity was observed for the compounds possessing nitro, chloro, methoxy, hydroxyl groups as substituent. Increase the length of the chain diminished the analgesic activity. Presence of fused heterocyclic ring provides good analgesic activity than single ring system.

2.3.4. Antimicrobial activity

Some of the compounds in the series **3a**-**l** showed promising antimicrobial activity against *E*scherichia *coli* and *B*acillus *subtilis*. Compounds **3b** and **3h** showed good inhibition at 31.2 μg/mL against *E. coli* and **3e** and **3h** showed good inhibition at the same concentration against *B. subtilis*. Compounds **3e**, **3f**, **3h** and **3i** showed good inhibition against *P*seudomonas *aeruginosa*, *S*taphylococcus *aureus*, *Aspergillus niger* and *C*andida *albicans*. Among this series **3h** was the most active one, since it shows good inhibition against all the tested bacterial and fungal strains except *P. aeruginosa*.

3. Conclusion

In conclusion, a series of pyrimidine analogs of indane-1,3-dione were synthesized in good yields. From docking studies it was true that the compound **3e** display a high degree of selectivity towards COX-2 than COX-1. It was further proved by its pharmacological activity. In addition to this binding with Pro 218 could be the possible reason for its good anti-inflammatory activity. This binding was not observed in any other derivatives. This clearly indicates that, binding with specific amino acids causes good confirmation change in the protein structure, there by good pharmacological effect was observed. Since the same compound possess good analgesic and antimicrobial activity the product was worthy for additional studies in view of their therapeutic potential for further investigation and derivatization for the development of a new class of drugs in a single component.

4. Experimental

Melting point was determined by conventional melting point apparatus and was uncorrected. The progress of the reaction was monitored on readymade silica gel plates (Merck). Infrared spectra were recorded by FT-IR technique using Tensor 27 spectrophotometer, Bruker Optic (Germany) by ATR technique. ^{1}H NMR spectra of synthesized compounds were recorded using AMX-400 at 400 MHz using DMSO as solvent. Mass spectra was recorded using Qstar Elite, LC MS. Animal experimentation was approved by IAEC of Acharya & B.M. Reddy College of Pharmacy.

4.1. Chemistry

4.1.1. Synthesis of chalcones derivatives of 2-acetylindane-1,3-dione

A mixture of 2-acetylindane-1,3-dione (0.01 mol) and substituted aromatic aldehyde (0.01 mol) were stirred in ethanol (25 mL) and then an aqueous solution of sodium hydroxide (10 mL) was added to it. The mixture was then kept overnight at room temperature and then poured into crushed ice. The solution was acidified with dilute hydrochloric acid. The chalcone derivatives of indane-1,3-dione precipitates out [34]. The product was filtered and recrystallized form ethanol.

*4.1.2. Synthesis of 2-(2-oxo-6-substituted-1,2,5,6-tetrahydro pyrimidin-4-yl)indane-1,3-dione (**3a**-**l**)*

A mixture of chalcones **2a**-**l** (0.01 mol), urea (0.01 mol), and sodium hydroxide (0.025 mol) in ethanol (25 mL) were refluxed for 6 h. The reaction mixture was cooled and acidified with dilute hydrochloric acid. The precipitate formed was filtered and recrystallized form ethanol to give **3a**-**l**.

*4.1.2.1. 2-(2-oxo-6-phenyl-1,2,5,6-tetrahydropyrimidin-4-yl)-indane-1,3-dione (**3a**).* Yield 59%; mp 182–185 °C; IR (cm^{-1}): 3226 (N–H stretching), 3021 (C–H aromatic stretching), 2944 (C–H aliphatic stretching), 1689 (C=O stretching), 1642 (C=N stretching), 1587 (C=C stretching); ^{1}H NMR δ (ppm): 10.821 (s, IH, NH), 7.422–7.438 (m, 2H), 7.456–7.502 (m, 2H) 7.653–7.675 (m, 1H), 7.678–7.689 (m, 2H),7.792–8.059 (m, 2H), 6.023–6.063 (dd, J = 9.6 Hz, J = 4.4 Hz, 1H), 3.421 (s, 1H), 2.210–2.253 (dd, J = 4.4 Hz, J = 11.2 Hz, 1H), 1.932–1.986 (dd, J = 11.2 Hz, J = 9.6 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-*d*6) δ 210.59, 209.98, 160.83, 137.84, 136.74, 136.41, 127.61, 129.41, 129.01, 128.34, 128.14, 127.37, 124.41, 123.31, 123.02, 119.43, 65.43, 57.85, 36.44; MS (*m*/*z*) (%): 318 [M$^+$, 12%]. Anal. Calcd for $C_{19}H_{14}N_2O_3$: C, 71.69; H, 4.43; N, 8.80. Found: C, 71.34; H, 4.33; N, 8.53.

*4.1.2.2. 2-[6-(2-nitrophenyl)-2-oxo-1,2,5,6-tetrahydropyrimidin-4-yl]-indane-1,3-dione (**3b**).* Yield 60%; mp 166–167 °C; IR (cm^{-1}): 3283 (N–H stretching), 3044 (C–H aromatic stretching), 2941 (C–H aliphatic stretching), 1698 (C=O stretching), 1633 (C=N stretching), 1588 (C=C stretching); ^{1}H NMR δ (ppm): 10.621 (s, IH, NH), 7.047–7.098 (m, 2H), 7.135–7.236 (m, 2H) 7.352–7.395 (m, 1H), 7.528–7.692 (m, 2H),7.923–8.981 (m, 1H), 6.211–6.261 (dd, J = 10.4 Hz, J = 5.2 Hz, 1H), 3.351 (s, 1H), 2.436–2.486 (dd, J = 5.2 Hz, J = 12.8 Hz, 1H), 1.852–1.859 (dd, J = 12.8 Hz, J = 10.4 Hz, 1H); MS (*m*/*z*) (%): 362 [M$^+$ − 1, 34%]. Anal. Calcd for $C_{19}H_{13}N_3O_5$: C, 62.81; H, 3.61; N, 11.57. Found: C, 62.55; H, 3.23; N, 11.12.

*4.1.2.3. 2-[6-(3-nitrophenyl)-2-oxo-1,2,5,6-tetrahydropyrimidin-4-yl]-indane-1,3-dione (**3c**).* Yield 62%; mp 173–174 °C; IR (cm^{-1}): 3346.59 (N–H stretching), 3002 (C–H aromatic stretching), 2881 (C–H aliphatic stretching), 1701 (C=O stretching), 1619 (C=N

stretching), 1583 (C=C stretching); ^{1}H NMR δ (ppm): 10.203 (s, IH, NH), 7.315–7.385 (m, 2H), 7.402–7.466 (m, 2H) 7.523–7.599 (m, 1H), 7.652–7.792 (m, 2H), 7.899–7.974 (m, 1H),7.315–7.974 (m, 8H, aromatic), 6.410–6.452 (dd, J = 10.0 Hz, J = 5.6 Hz, 1H), 3.321 (s, 1H), 2.501–2.550 (dd, J = 5.6 Hz, J = 12.4 Hz, 1H), 1.842–1.895 (dd, J = 12.4 Hz, J = 10.0 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-*d*6) δ 210.64, 209.87, 160.75, 137.17, 136.72, 136.42, 127.65, 127.38, 125.12, 125.14, 124.44, 123.34, 123.15, 120.11, 119.35, 115.48, 65.41, 57.97, 36.58; MS (*m*/*z*) (%): 363 [M^+, 23%]. Anal. Calcd for $C_{19}H_{13}N_3O_5$: C, 62.81; H, 3.61; N, 11.57. Found: C, 62.58; H, 3.24; N, 11.22.

*4.1.2.4. 2-[6-(4-nitrophenyl)-2-oxo-1,2,5,6-tetrahydropyrimidin-4-yl]-indane-1,3-dione (**3d**).* Yield 43%; mp 182–183 °C; IR (cm^{-1}): 3362 (N–H stretching), 3000 (C–H aromatic stretching), 2901 (C–H aliphatic stretching), 1698 (C=O stretching), 1621 (C=N stretching), 1582 (C=C stretching); ^{1}H NMR δ (ppm): 10.181 (s, IH, NH), 7.021–7.152 (m, 2H), 7.256–7.351 (m, 2H) 7.482–7.586 (m, 1H), 7.681–7.735 (m, 2H), 7.832–7.952 (m, 1H), 7.021–7.952 (m, 8H, aromatic), 6.251–6.294 (dd, J = 10.0 Hz, J = 5.2 Hz, 1H), 3.494 (s, 1H), 2.632–2.674 (dd, J = 5.2 Hz, J = 12.0 Hz, 1H), 1.848–1.896 (dd, J = 12.0 Hz, J = 10.0 Hz, 1H); MS (*m*/*z*) (%): 364 [M^+ + 1, 22%]. Anal. Calcd for $C_{19}H_{13}N_3O_5$: C, 62.81; H, 3.61; N, 11.57. Found: C, 62.63; H, 3.23; N, 11.11.

*4.1.2.5. 2-[6-(4-chlorophenyl)-2-oxo-1,2,5,6-tetrahydropyrimidin-4-yl]-indane-1,3-dione (**3e**).* Yield 56%; mp 176–178 °C; IR (cm^{-1}): 3328.57 (N–H stretching), 3192 (C–H aromatic stretching), 2921 (C–H aliphatic stretching), 1702 (C=O stretching), 1640 (C=N stretching), 1562 (C=C stretching); ^{1}H NMR δ (ppm): 10.901 (s, IH, NH), 6.932–7.052 (m, 2H), 7.159–7.256 (m, 2H) 7.361–7.422 (m, 1H), 7.582–7.627 (m, 2H), 7.698–7.785 (m, 1H), 6.402–6.452 (dd, J = 9.6 Hz, J = 5.6 Hz, 1H), 3.521 (s, 1H), 2.342–2.684 (dd, J = 5.6 Hz, J = 11.2 Hz, 1H), 1.622–4.672 (dd, J = 11.2 Hz, J = 9.6 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-*d*6) δ 210.69, 209.74, 160.77, 136.84, 136.75, 135.78, 127.68, 127.34, 127.15, 126.01, 125.37, 123.48, 123.31, 123.01, 120.98, 119.48, 65.47, 57.88, 36.47; MS (*m*/*z*) (%) : 354 [M^+ + 2, 10%]. Anal. Calcd for $C_{19}H_{13}ClN_2O_3$: C, 64.69; H, 3.71; N, 7.94. Found: C, 64.52; H, 3.55; N, 7.46.

*4.1.2.6. 2-{6-[4-(dimethylamino)phenyl]-2-oxo-1,2,5,6-tetrahydropyrimidin-4-yl}-indane-1,3-dione (**3f**).* Yield 59%; mp 162–163 °C; IR (cm^{-1}): 3312.41 (N–H stretching), 3055 (C–H aromatic stretching), 2912 (C–H aliphatic stretching), 1662 (C=N stretching), 1591 (C=C stretching); ^{1}H NMR δ (ppm): 9.674 (s, 1H, NH), 7.045–7.154 (m, 2H), 7.232–7.366 (m, 2H) 7.488–7.592 (m, 1H), 7.601–7.652 (m, 2H), 7.689–7.724 (m, 1H), 6.693–6.767 (dd, J = 9.8 Hz, J = 5.2 Hz, 1H), 3.384 (s, 1H), 2.970–3.045 (dd, J = 5.2 Hz, J = 12.4 Hz, 1H), 2.516 (s, 6H), 2.315–2.392 (dd, J = 12.2 Hz, J = 9.8 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-*d*6) δ 210.57, 209.87, 160.81, 136.41, 136.21, 135.80, 127.57, 127.47, 127.27, 126.01, 125.57, 123.54, 123.21, 123.07, 120.88, 119.48, 65.44, 57.74, 36.44, 28.37, 28.21; MS (*m*/*z*) (%): 362 [M^+ + 1, 11%]. Anal. Calcd for $C_{21}H_{19}N_3O_3$: C, 69.79; H, 5.30; N, 11.63. Found: C, 69.56; H, 5.14; N, 11.42.

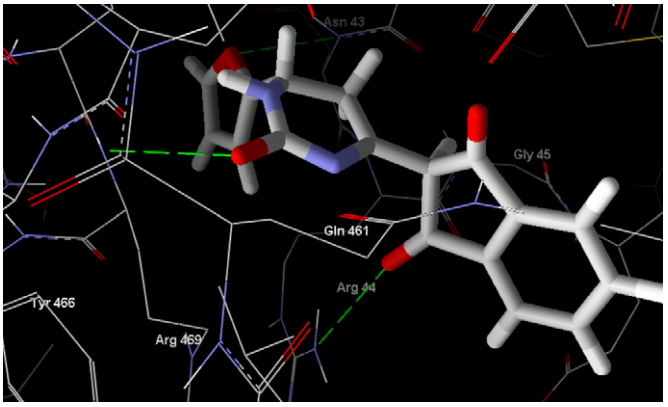


Fig. 2. Binding mode of **3h** into the binding site of **1CX2**.

*4.1.2.7. 2-[6-(4-methoxyphenyl)-2-oxo-1,2,5,6-tetrahydropyrimidin-4-yl]-indane-1,3-dione (**3g**).* Yield 64%; mp 210–212 °C; IR (cm^{-1}): 3355 (N–H stretching), 3071 (C–H aromatic stretching), 2881 (C–H aliphatic stretching), 1702 (C=O stretching), 1642 (C=N stretching), 1586 (C=C stretching); ^{1}H NMR δ (ppm): 9.951 (s, 1H, NH), 7.414–7.472 (m, 2H), 7.552–7.599 (m, 2H) 7.621–7.665 (m, 1H), 7.682–7.702 (m, 2H), 7.705–7.751 (m, 1H), 6.521–6.565 (dd, J = 10.4 Hz, J = 5.2 Hz, 1H), 3.401 (s, 1H), 3.157 (s, 3H), 2.632–2.685 (dd, J = 5.2 Hz, J = 12.4 Hz, 1H), 2.052–2.096 (dd, J = 12.4 Hz, J = 10.4 Hz, 1H); MS (*m*/*z*) (%): 349 [M^+ + 1, 21%]. Anal. Calcd for $C_{20}H_{16}N_2O_4$: C, 68.98; H, 4.63; N, 8.04. Found: C, 38.66; H, 4.52; N, 8.22.

*4.1.2.8. 2-[6-(2-furyl)-2-oxo-1,2,5,6-tetrahydropyrimidin-4-yl]-indane-1,3-dione (**3h**).* Yield 69%; mp 145–146 °C; IR (cm^{-1}): 3359 (N–H stretching), 3064 (C–H aromatic stretching), 2882 (C–H aliphatic stretching), 1701 (C=O stretching), 1620 (C=N stretching), 1586 (C=C stretching); ^{1}H NMR δ (ppm): 10.382 (s, IH, NH), 7.211–7.252 (m, 2H), 7.256–7.358 (m, 2H) 7.452–7.505 (m, 1H), 7.658–7.765 (m, 2H) 6.820–6.868 (dd, J = 9.6 Hz, J = 5.6 Hz, 1H), 3.504 (s, 1H), 2.401–2.452 (dd, J = 5.6 Hz, J = 11.6 Hz, 1H), 2.056 (dd, J = 11.6 Hz, J = 9.4 Hz, 1H); MS (*m*/*z*) (%): 309 [M^+ + 1, 12%]. Anal. Calcd for $C_{17}H_{12}N_2O_4$: C, 66.23; H, 3.92; N, 9.09. Found: C, 66.05; H, 3.59; N, 8.95.

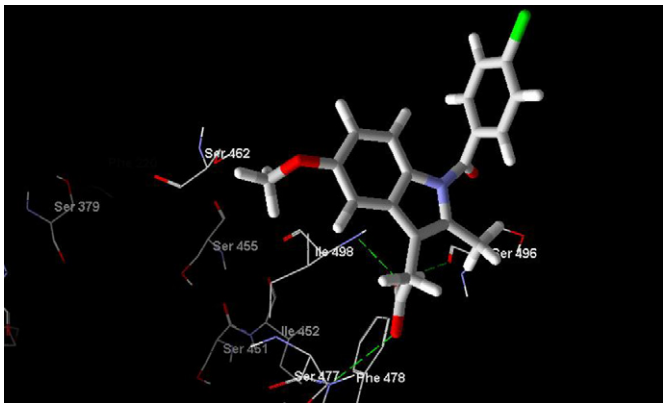


Fig. 1. Binding mode of indomethacin into the binding site of **1CX2**.

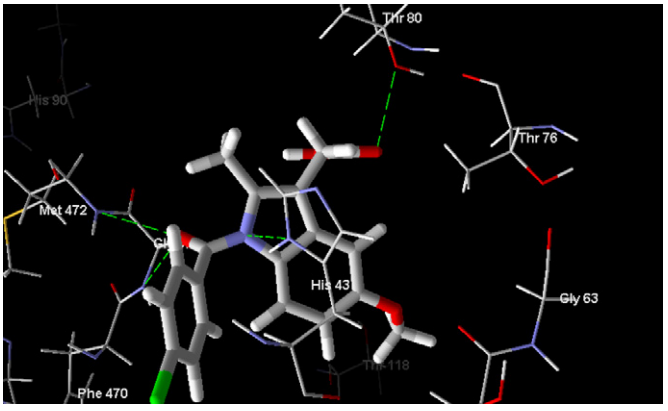


Fig. 3. Binding mode of indomethacin into the binding site of **2OYE**.

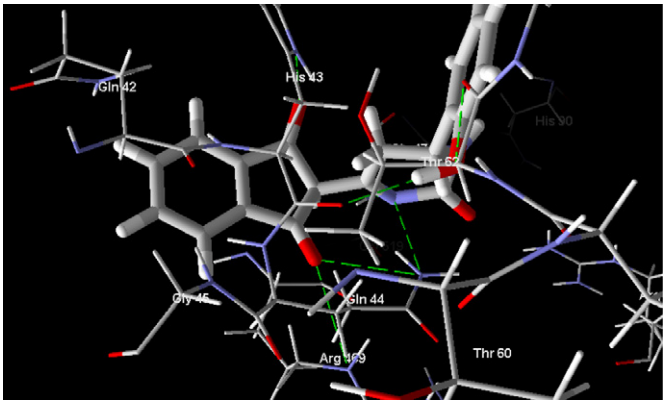


Fig. 4. Binding mode of **4j** into the binding site of **2OYE**.

*4.1.2.9. 2-{2-oxo-6-[2-phenylvinyl]-1,2,5,6-tetrahydropyrimidin-4-yl}-indane-1,3-dione (**3i**).* Yield 49%; mp 186–187 °C; IR (cm^{-1}): 3394 (N–H stretching), 3085 (C–H aromatic stretching), 2863 (C–H aliphatic stretching), 1692 (C=O stretching), 1642 (C=N stretching), 1544 (C=C stretching); ^{1}H NMR δ (ppm): 10.052 (s, IH, NH), 6.932–7.052 (m, 2H), 7.122–7.252 (m, 2H) 7.289–7.321 (m, 1H), 7.356–7.452 (m, 2H), 7.521–7.602 (m, 2H), 7.952–8.021 (m, 2H), 6.851–6.898 (dd, J = 10.8 Hz, J = 5.2 Hz, 1H), 3.402 (s, 1H), 2.652–2.699 (dd, J = 5.2 Hz, J = 12.8 Hz, 1H), 1.921–1.970 (dd, J = 12.8 Hz, J = 10.4 Hz, 1H); MS (m/z) (%): 344 [M^+, 22%]. Anal. Calcd for $C_{21}H_{16}N_2O_3$: C, 73.24; H, 4.68; N, 8.13. Found: C, 73.02; H, 4.52; N, 8.08.

*4.1.2.10. 2-[6-(2-hydroxyphenyl)-2-oxo-1,2,5,6-tetrahydropyrimidin-4-yl]-indane-1,3-dione (**3j**).* Yield 58%; mp 189–191 °C; IR (cm^{-1}): 3373 (O–H stretching), 3361 (N–H stretching), 3083 (C–H aromatic stretching), 2925 (C–H aliphatic stretching), 1641 (C=O stretching), 1641 (C=N stretching), 1542 (C=C stretching); ^{1}H NMR δ (ppm): 12.152 (s, 1H, OH), 9.942 (s, IH, NH), 7.321–7.388 (m, 2H), 7.402–7.483 (m, 2H) 7.525–7.608 (m, 1H), 7.898–8.086 (m, 2H) 8.126–8.289 (m, 1H), 7.105–7.152 (dd, J = 10.4 Hz, J = 4.8 Hz, 1H), 3.478 (s, 1H), 2.654–2.692 (dd, J = 4.8 Hz, J = 12.0 Hz, 1H), 2.458–2.499 (dd, J = 12.0 Hz, J = 10.4 Hz, 1H); MS (m/z) (%): 335 [M^+ + 1, 12%]. Anal. Calcd for $C_{19}H_{14}N_2O_4$: C, 68.26; H, 4.22; N, 8.38. Found: C, 68.06; H, 4.12; N, 8.12.

*4.1.2.11. 2-[6-(1H-indol-2-yl)-2-oxo-1,2,5,6-tetrahydropyrimidin-4-yl]-indane-1,3-dione (**3k**).* Yield 59%; mp 110–112 °C; IR (cm^{-1}): 3373 (N–H stretching), 3083 (C–H aromatic stretching), 2989 (C–H aliphatic stretching), 1702 (C=O stretching), 1620 (C=N stretching), 1586 (C=C stretching); ^{1}H NMR δ (ppm): 12.142 (s, IH, NH), 10.025 (s, IH, NH), 7.211–7.302 (m, 2H), 7.352–7.415 (m, 2H) 7.424–7.501 (m, 1H), 7.689–7.756 (m, 2H) 7.852–7.921 (m, 1H), 8.226–8.306 (m, 1H), 6.761–6.810 (dd, J = 9.4 Hz, J = 5.4 Hz, 1H), 3.125 (s, 1H), 2.610–2.690 (dd, J = 5.4 Hz, J = 11.8 Hz, 1H), 2.462–2.502 (dd, J = 11.8 Hz, J = 9.4 Hz, 1H); MS (m/z) (%): 358 [M^+ + 1, 23%]. Anal. Calcd for $C_{21}H_{15}N_3O_3$: C, 70.58; H, 4.23; N, 11.76. Found: C, 70.52; H, 4.13; N, 11.59.

*4.1.2.12. 2-[6-(4-hydroxy-3-methoxyphenyl)-2-oxo-1,2,5,6-tetrahydropyrimidin-4-yl]-indane-1,3-dione (**3l**).* Yield 53%; mp 165–168 °C; IR (cm^{-1}): 3545 (O–H stretching) 3331 (N–H stretching), 3034 (C–H aromatic stretching), 2942 (C–H aliphatic stretching), 1709 (C=O stretching), 1641 (C=N stretching), 1576 (C=C stretching); ^{1}H NMR δ (ppm): 11.212 (s, IH, OH), 10.211 (s, IH, NH), 6.955–7.121 (m, 2H), 7.221–7.311 (m, 2H) 7.426–7.521 (m, 1H), 7.625–7.721 (m, 1H) 7.921–8.052 (m, 1H), 6.754–6.822 (dd, J = 10.4 Hz, J = 5.2 Hz, 1H), 3.411 (s, 1H), 3.221 (s, 3H) 2.521–2.593 (dd, J = 5.2 Hz, J = 12.4 Hz, 1H), 2.221–2.286 (dd, J = 12.4 Hz, J = 10.4 Hz, 1H; MS (m/z) (%): 364 [M^+, 22%). Anal. Calcd for $C_{20}H_{16}N_2O_5$: C, 65.93; H, 4.43; N, 7.69. Found: C, 65.65; H, 4.29; N, 7.49.

4.2. Docking studies

Molecular docking studies of the synthesized compounds were performed in order to rationalize the obtained biological results. Molecular docking studies further help in understanding the various interactions between the ligand and enzyme active site in detail.

Docking study was carried out for the target compounds using Molegro Virtual docker version 2010. MolDock scoring function is used by MVD program is defined by

$$E_{score} = E_{inter} + E_{intra}$$

Where, E_{score} = MolDock score.

E_{inter} = ligand–Protein interaction
E_{intra} = internal energy of the ligand.

The ligand structure was drawn in Marvin Sketch 5.4. Hydrogen atoms were viewed. The 2D structure was then converted into 3D and saved as PDB file type. It was then imported into Molegro Virtual Docker. The Pdb codes **1CX2** and **2OYE** were downloaded from the protein data bank www.pdb.org then docking was carried

Table 1
Anti-inflammatory activity of the synthesized compounds.

Compound	Change in paw volume (in mL) after drug administration (Mean ± SEM[a])				Percentage inhibition of edema volume after			
	1 h	2 h	3 h	4 h	1 h	2 h	3 h	4 h
Control	0.76 ± 0.03	0.83 ± 0.02	0.66 ± 0.02	0.60 ± 0.02	–	–	–	–
Standard	0.58 ± 0.06*	0.43 ± 0.02**	0.41 ± 0.02**	0.39 ± 0.02*	23	48	39	34
3a	0.61 ± 0.04	0.55 ± 0.03**	0.52 ± 0.03	0.53 ± 0.03	20	33	21	11
3b	0.68 ± 0.05	0.58 ± 0.03**	0.49 ± 0.05*	0.41 ± 0.03*	11	30	26	32
3c	0.56 ± 0.06*	0.53 ± 0.03**	0.53 ± 0.03	0.52 ± 0.03	27	36	21	12
3d	0.64 ± 0.02	0.61 ± 0.05**	0.53 ± 0.06	0.49 ± 0.06	16	26	19	18
3e	0.64 ± 0.03	0.68 ± 0.06	0.46 ± 0.03**	0.39 ± 0.04*	15	19	31	34
3f	0.65 ± 0.02	0.59 ± 0.03**	0.53 ± 0.03	0.50 ± 0.05	15	29	20	16
3g	0.60 ± 0.05	0.66 ± 0.03	0.51 ± 0.04	0.54 ± 0.04	22	21	22	10
3h	0.63 ± 0.06	0.62 ± 0.02**	0.53 ± 0.03	0.56 ± 0.02	18	25	20	7
3i	0.63 ± 0.07**	0.57 ± 0.02**	0.55 ± 0.03	0.50 ± 0.04	17	32	18	16
3j	0.58 ± 0.04	0.65 ± 0.05*	0.54 ± 0.03	0.50 ± 0.05	24	22	19	16
3k	0.62 ± 0.03	0.66 ± 0.07	0.58 ± 0.02	0.52 ± 0.06	19	21	12	12
3l	0.61 ± 0.05	0.56 ± 0.02**	0.49 ± 0.03*	0.53 ± 0.04	20	33	26	11

*P < 0.05; **P < 0.01; ***P < 0.001.
[a] Standard Error Mean.

out for the structural proteins [35]. Figs. 1–4 represent the binding modes of indomethacin, **3h** and **4j** with **1CX2** and **2OYE**.

4.3. Biological activities

4.3.1. Anti-inflammatory activity

Rats weighing between 150–200 g were selected randomly (excluding pregnant female rats) for the test [12]. Animals were housed with food and water for two days before testing. A mark was made on hind paw just beyond tibiotarsal junction, to ensure constant paw volume. The initial paw volume of each rat was noted by mercury displacement method. Animals were divided into groups with six animals in each group. The first groups of rats were treated with 0.1 mL of 1% carboxy methylcellulose suspension orally (control). The second group was administered with at a dose of 10 mg/kg of the suspension of indomethacin (standard) and the other groups were treated with 10 mg/kg of the suspension of test compounds. After 30 min 0.1 mL of 1% (w/v) carrageenan was injected in the plantar region of the left paw of control, standard and test groups. The paw volumes were noted for 1, 2, 3 and 4 h after carrageenan challenge. The percent difference in paw volumes were calculated and expressed as percent edema inhibition by the drugs. It was calculated by using the formula

$$1 - \frac{Vt}{Vc} \times 100$$

Where

Vt: – edema volume in drug treated group.
Vc: – edema volume in the control group.

The results of anti-inflammatory activity of the synthesized compounds were specified in Table 1.

4.3.2. Ulcerogenic effect

Ulceration in rats was induced as described by Goyal et al. Wistar albino rats weighing 150–200 g of either sex were divided into various groups of six animals. Control group of animals were treated with 10% v/v Tween 80 suspension intraperitoneally [12]. One group was administered with indomethacin intraperitoneally in a dose of 10 mg/kg once daily for three days. The remaining group was administered with test compounds intraperitoneally at the dose of 10 mg/kg. On the fourth day, pylorus was ligated and the animals were fasted for 36 h before the ligation procedure. Four hours after ligation, animals were sacrificed. The stomach was removed along with the greater curvature. Ulcer index was determined. The results were reported in Table 2.

4.3.3. Analgesic activity

The mice were weighed and numbered. Animals were divided into different groups, each containing at least six mice [12]. Appropriate volume of acetic acid solution was administered to the first group (which serves as control) and placed individually under glass jar for observation. The onset on writhes was noted. The number of abdominal contractions, trunk twist response and extension of hind limbs as well as the number of animals showing such response during a period of 10 min was recorded. The standard and test compounds were injected to the other group of animals at the dose of 10 mg/kg. Fifteen minutes later, acetic acid solution was administered to these animals. The onset and severity of writhing response were noted. The mean writhing scores in control and compound treated groups were calculated and inhibition of pain response by compounds was noted.

$$\text{Percentage inhibition of pain} = 1 - \frac{Vt}{Vc} \times 100$$

Where,

Vt: – number of writhing (mean) in drug treated group.
Vc: – number of writhing (mean) in control group.

The results of analgesic activity of synthesized compounds were tabulated in Table 3.

4.3.4. Antimicrobial activity

Minimum inhibitory concentration (MIC) of any compound is defined as the lowest concentration, which completely inhibits growth (turbidity on liquid media). *In vitro* antimicrobial activities of the compounds were tested by using the tube dilution method. The medium was prepared and the sterile broth was then poured in each sterile tube followed by addition of compound in first tube. Two-fold serial dilution was carried out from the tubes and excess broth was discarded from the last tube. McFarland standard was compared visually to a microbial suspension of

Table 2
Evaluation of ulcerogenicity index.

Compound	Ulcer index (Mean ± SEM[a])
Control	0.13 ± 0.22
Indomethacin	1.70 ± 0.32**
3e	1.28 ± 0.32*

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
[a] Standard Error Mean.

Table 3
Analgesic activity of the synthesized compounds.

Compound	No. of writhing in 15 min (Mean ± SEM[a])	Percentage inhibition of pain
Control	36.20 ± 0.84	–
Standard	16.50 ± 1.18**	55
3a	25.33 ± 1.76**	31
3b	23.83 ± 1.13**	35
3c	23.33 ± 1.56**	36
3d	22.66 ± 0.98**	38
3e	20.50 ± 2.09**	44
3f	17.50 ± 1.20**	52
3g	25.33 ± 1.89**	31
3h	27.66 ± 1.90*	25
3i	30.83 ± 1.97	16
3j	23.00 ± 2.11**	37
3k	29.00 ± 1.77*	21
3l	26.5 ± 1.33**	28

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
[a] Standard Error Mean.

Table 4
Antimicrobial activity of the synthesized compounds.

Compound	Minimum inhibitory concentration (μg/mL)					
	E. coli	*P. aeruginosa*	*B. subtilis*	*S. aureus*	*A. niger*	*C. albicans*
Ampicillin	3.9	3.9	3.9	3.9	–	–
Fluconazole	–	–	–	–	7.8	7.8
3a	62.5	125	250	125	500	500
3b	31.2	250	250	125	500	500
3c	250	250	250	125	125	500
3d	500	250	250	125	500	500
3e	125	62.5	31.2	125	125	125
3f	125	62.5	125	125	500	500
3g	250	250	125	500	250	250
3h	31.2	500	31.2	62.5	62.5	62.5
3i	125	250	125	62.5	250	250
3j	500	125	250	125	500	250
3k	250	125	250	250	125	500
3l	500	250	250	125	125	250

approximately 1.5×10^8 cells/mL. To each tube, standard inoculums were added [36].

The test compounds, ampicillin and fluconazole were diluted to get 1000, 500, 250, 125, 62.5, 31.2, 15.6, 7.8, 3.9, 1.9, 0.97 μg/mL concentration. 10% DMSO was used as a solvent. All determinations were done in duplicate and the average was taken as final reading. In order to ensure that the solvent had no effect on the growth a control test was performed containing inoculated broth suspended with 10% DMSO. Then it was kept for incubation.

4.3.4.1. Antibacterial. The antibacterial activities of the synthesized compounds were tested against *E. coli* (MTCC-4351), *P. aeruginosa*, (MTCC-424), *B. subtilis*, (MTCC-441) and *S. aureus* (MTCC-3160) using Muller–Hinton medium. The pH was maintained at 7.4. The incubation was carried out at 37 ± 1 °C for 24 h. After incubation for 24 h, the tube with no growth of microorganism was recorded to represent MIC, which was expressed in μg/mL. At the end of the incubation period, the MIC values were determined. The results were reported in Table 4.

4.3.4.2. Antifungal activities. The antifungal activities of the synthesized compounds were tested against *A. niger* (MTCC-282) and *C. albicans* (MTCC-183) using sabouraud dextrose broth maintained at pH 7.4. The tubes were incubated at 25 ± 1 °C for 48 h. The tube with no growth of fungi was recorded to represent MIC expressed in μg/mL. The results were reported in Table 4.

Acknowledgment

Authors are thankful to Sri. B. Premnath Reddy, Chairman, Acharya Institutes for providing facilities for the successful completion of the project. We also thank Dr. Rene Thomsen for providing Molegro trial license.

References

[1] V. Vassileva, M. Piquette-Miller, Clin. Pharmacol. Ther. 87 (2010) 375–379.
[2] A. Lanas, Dig. Liver Dis. 33 (2001) S29–S34.
[3] B.M.D.F. Peter, Am. J. Med. 104 (1998) 9S–13S.
[4] P. David A, Am. J. Med. 117 (2004) S63–S71.
[5] F. Buttgereit, G.R. Burmester, L.S. Simon, Am. J. Med. 110 (2001) S13–S19.
[6] M. Denis, Am. J. Med. 105 (1998) 3S–9S.
[7] C. Charlier, C. Michaux, Eur. J. Med. Chem. 38 (2003) 645–659.
[8] X.-P. Miao, Q. Ouyang, H.-Y. Li, Z.-H. Wen, D.-K. Zhang, X.-Y. Cui, Curr. Ther. Res. 69 (2008) 181–191.
[9] S. Ronald S, Semin. Arthritis Rheum. 26 (1996) 435–446.
[10] B. Cryer, M. Feldman, Am. J. Med. 104 (1998) 413–421.
[11] A.A. Abu-Hashem, M.M. Youssef, Molecules 16 (2011) 1956–1972.
[12] V. Alagarsamy, S. Meena, K.V. Ramseshu, V.R. Solomon, K. Thirumurugan, K. Dhanabal, M. Murugan, Eur. J. Med. Chem. 41 (2006) 1293–1300.
[13] K.M. Amin, M.M. Hanna, H.E. Abo-Youssef, R.F. George, Eur. J. Med. Chem. 44 (2009) 4572–4584.
[14] N. Kumar, A. Chauhan, S. Drabu, Biomed. Pharmacother. 65 (2011) 375–380.
[15] G. Amr Ael, H.H. Sayed, M.M. Abdulla, Arch. Pharm. 338 (2005) 433–440.
[16] R.S. Keri, K.M. Hosamani, R.V. Shingalapur, M.H. Hugar, Eur. J. Med. Chem. 45 (2010) 2597–2605.
[17] N.M. Abunada, H.M. Hassaneen, N.G. Kandile, O.A. Miqdad, Molecules 13 (2008) 1501–1517.
[18] E.S. Al-Abdullah, A.R. Al-Obaid, O.A. Al-Deeb, E.E. Habib, A.A. El-Emam, Eur. J. Med. Chem. 46 (2011) 4642–4647.
[19] G. Auzzi, A. Costanzo, F. Bruni, M. Clauser, G. Guerrini, S. Selleri, L. Pecori Vettori, Farmaco 45 (1990) 1193–1205.
[20] A.A. Bekhit, H.T.Y. Fahmy, S.A.F. Rostom, A.M. Baraka, Eur. J. Med. Chem. 38 (2003) 27–36.
[21] O.M. Ahmed, M.A. Mohamed, R.R. Ahmed, S.A. Ahmed, Eur. J. Med. Chem. 44 (2009) 3519–3523.
[22] A.G. Amr, A.M. Mohamed, S.F. Mohamed, N.A. Abdel-Hafez, F. Hammam Ael, Bioorg. Med. Chem. 14 (2006) 5481–5488.
[23] M. Debi, Indian J. Exp. Biol. 35 (1997) 1208–1213.
[24] A. Kamal, J.S. Reddy, M.J. Ramaiah, E.V. Bharathi, D. Dastagiri, M.K. Reddy, S.N. Pushpavalli, M. Pal-Bhadra, Bioorg. Med. Chem. Lett. 20 (2010) 5232–5236.
[25] S. Kim, D.H. Park, T.H. Kim, M. Hwang, J. Shim, FEBS J. 276 (2009) 4715–4726.
[26] D. Giles, M. Prakash, K. Ramsesu, E-J Chem 4 (2007) 428–433.
[27] W. He, F.-C. Huang, B. Hanney, J. Souness, B. Miller, G. Liang, J. Mason, S. Djuric, J. Med. Chem. 41 (1998) 4216–4223.
[28] S. Robert-Piessard, D. Leblois, P. Kumar, J. Robert, G.L. Baut, L. Sparfel, B.R.E.K.R. Sanchez, J. Petit, L. Welin, Eur. J. Med. Chem. 25 (1990) 735–747.
[29] P. Crooks, R. Sommeerville, J. Pharm. Sci. 71 (1982) 291–294.
[30] S.L. Shapiro, K. Geiger, L. Freedman, J. Org. Chem. 25 (1960) 1860–1865.
[31] S. Jubie, S. Meena, K.V. Ramaseshu, N. Jawahar, S. Vijayakumar. Sect. B, Indian J. Chem. 49 (2010) 1261–1263.
[32] S. Inayama, K. Mamoto, T. Shibata, M. Hirose, J. Med. Chem. 19 (1976) 433–436.
[33] T. Matsuo, T. Takagi, K. Saito, H. Yuki, T. Yamaguchi, Yakugaku Zasshi 101 (1981) 960–964.
[34] Y. Rajendraprasad, A. Laxshnamarao, R. Rambabu, E-J Chem 5 (2008) 461–466.
[35] B. Sandhya, D. Giles, V. Mathew, G. Basavarajaswamy, R. Abraham, Eur. J. Med. Chem. 46 (2011) 4696–4701.
[36] J.M. Andrews, J. Antimicrob. Chemother. 48 (suppl. S1) (2001) 5–16.