Abstract: Ten benzoazole clubbed 2-pyrrolidinones (11–20) as human monoacylglycerol lipase inhibitors were designed on the criteria fulfilling the structural requirements and on the basis of previously reported inhibitors. The designed, synthesized, and characterized compounds (11–20) were screened against monoacylglycerol lipase (MAGL) in order to find potential inhibitors. Compounds 19 (4-NO₂ derivative) and 20 (4-SO₂NH₂ derivative), with an IC₅₀ value of 8.4 and 7.6 nM, were found most active, respectively. Both of them showed micromolar potency (IC₅₀ value above 50 μM) against a close analogue, fatty acid amide hydrolase (FAAH), therefore considered as selective inhibitors of MAGL. Molecular docking studies of compounds 19 and 20 revealed that carbonyl of 2-pyrrolidinone moiety sited at the oxyanion hole of catalytic site of the enzyme stabilized with three hydrogen bonds (~2 Å) with Ala51, Met123, and Ser122, the amino acid residues responsible for the catalytic function of the enzyme. Remarkably, the physiochemical and pharmacokinetic properties of compounds 19 and 20, computed by QikProp, were found to be in the qualifying range as per the proposed guideline for good orally bioactive CNS drugs. In formalin-induced nociception test, compound 20 reduced the pain response in acute and late stages in a dose-dependent manner. They significantly demonstrated the reduction in pain response, having better potency than the positive control gabapentin (GBP), at 30 mg/kg dose. Compounds 19 and 20 were submitted to NCI, USA, for anticancer activity screening. Compounds 19 (NSC: 778839) and 20 (NSC: 778842) were found to have good anticancer activity on SNB-75 cell line of CNS cancer, exhibiting 35.49 and 31.88% growth inhibition (% GI), respectively.

Keywords: analgesic; anticancer; pyrrolidin-2-one; benzoxazole; MAGL inhibitors; molecular docking

1. Introduction

Endocannabinoids (endogenous ligands), cannabinoid (CB) receptors, and proteins for their biological synthesis and degradation constitute the endocannabinoid system (ECS) [1]. Endocannabinoids are biosynthesized from the membrane phospholipids [2]. Endocannabinoid, N-arachidonoyl ethanolamine (AEA, Anandamide) functions as partial agonist on CB₁ and CB₂ receptors. It has low affinity for CB₂ and moderate affinity for CB₁. Endocannabinoids, 2-arachidonoylglycerol (2-AG) function as full agonist and have moderate affinity for both the receptors. Interestingly, 2-AG is the major endocannabinoid and is found to be approximately 170-fold higher in concentration than AEA, in the brain [3]. AEA and 2-AG hydrolysis and degradation are facilitated by fatty acid amid hydras.
(FAAH) and monoacylglycerol lipase (MAGL) enzymes, correspondingly [1]. MAGL (an α/β-hydrolase) hydrolyzes 2-AG into glycerol and free fatty acid (FFA), by the action of the catalytic triad organized with Ser122, Asp239 and His269 amino acids in the active site of the enzyme [4]. MAGL hydrolyzes approximately 85% of total 2-AG in the CNS [5] and generates arachidonic acid (AA), giving rise to neuro-inflammatory PGE2 and PGD2 prostaglandins [6]. MAGL inhibitors ameliorates neuropathic pain by increasing 2-AG and decreasing neuro-inflammatory prostaglandins in the CNS (Figure 1) [7]. In addition, 2-AG demonstrated analgesic activity by acting on CB1 receptors in the CNS and periphery [8–11]. It is reported that JJKK-048 (MAGL, IC50 363 pM) exhibited analgesic activity in tail immersion and writhing test [12]. MAGL inhibition has shown significant neuroprotective and anti-inflammatory potential in Parkinson’s and Alzheimer’s disease [13,14]. PF-06795071 (IC50 3 nM), a MAGL inhibitor, is reported to have considerable anti-neuroinflammatory potential [15]. Another MAGL inhibitor, ABX-1431 (IC50 14 nM) is under clinical trials for broad range of CNS disorders like Tourette syndrome [16]. Many more research findings encouraged that the inhibitors of MAGL has therapeutic potentials in pain and CNS disorders [17–20].

![Figure 1. Role of MAGL inhibitors in the alleviation of pain and cancer. PG: prostaglandins; PA: phosphatidic acid; LPA: lysophosphatidic acid; and S1P: sphingosine-1-phosphate.](image)

The governing importance of MAGL in abnormal lipolysis in cancer has been demonstrated [21]. MAGL was originally known for its lipolytic action on monoacylglycerols from stored triacylglycerols into glycerol and free fatty acids (FFA) [22]. Cancer cells utilizes this lipolytic pathways for their hastened proliferation [23]. The cancer-supporting action of MAGL is due to elevated FFA levels. This MAGL-FFA pathway promotes in vivo tumor growth by increasing FFA-derived oncogenic signaling lipids (PA, LPA, S1P and PGE2) [18]. These protumorigenic lipid mediators encourage tumor growth, angiogenesis, and metastasis in cancer (Figure 1) [24]. MAGL is reported to expressed vastly in aggressive type of cancer cells and is associated with pathogenesis, proliferation, and in vivo tumor growth. MAGL inhibition disrupts cancer cell proliferation, growth and metastasis [25–27]. The anticancer effect of MAGL inhibition in prostate cancer was totally abolished by cotreat-
ment with SR141716 (rimonabant; CB1 receptor antagonist) and fatty acids, signifying that amplified endocannabinoid action and reduced stock of FFA from MAGL inhibition is the reason behind antitumor effect [26].

MAGL inhibitors identified till date includes URB602 (IC50 28 µM) [28], CAY10499 (IC50 144 nM) [29], SPBO1403 (IC50 31 µM) [30], JZL184 (IC50 8 nM) [31], SAR629 (IC50 1.1 nM) [32], KML29 (IC50 3.6 nM) [33], ML30 (IC50 0.54 nM) [34], JJKK-048 (IC50 363 pm) [35], AXB1431 (IC50 14 nM) [16], R(3t) (IC50 3.6 nM) [36], PF-06795071 (IC50 3 nM) [15], a benzoylpiperidine derivative (IC50 80 nM) [37], and a benzisothiazolinone derivative (IC50 34.1 nM) [38]. JJKK-048 and KML29 both were reported to be highly selective MAGL inhibitors, and their selectivity is more than 10,000-fold over FAAH. [33,35]. In addition, 3D crystal structures of human MAGL enzyme were elucidated by X-ray crystallography, and are available on protein data bank [32,36,39,40]. A comprehensive description of MAGL crystal structures and inhibitors were reviewed [41]. Our group has also identified nanomolar MAGL inhibitors, viz. ZINC24092691 (IC50 10 nM), ZINC12863377 (IC50 39 nM), a ZINC24092691 analogue (IC50 6.5 nM), a thiazole-5-carboxylate derivative (IC50 37 nM), and a pyrrolidin-2-one linked benzimidazole derivative (compound 25; IC50 9.4 nM) [42–45].

In continuation of our work on MAGL inhibitors, we have designed novel 2-pyrrolidinone linked benzoxazole derivatives and screened them for analgesic and anticancer effects.

2. Results

The binding pattern of pyrrolidin-2-one derivatives (ZINC12863377, compound 25 and compound R-3t), and the basic structural requirement for MAGL inhibitors was kept in mind to design novel pyrrolidin-2-one linked benzoxazole derivatives (Figure 2). The route of synthesis of compounds (11–20) is presented in Figure 3.

2.1. Chemistry

Intermediate compound 1, (1-Benzyl-5-oxopyrrolidine-3-carboxylic acid), was successfully prepared by fusion of benzylamine and methylidenesuccinic acid in water, while compounds 2–10 were synthesized as reported by us in our previous publication [45].

The fusion of 1-substituted-5-oxopyrrolidine-3-carboxylic acids (1–10) with 2-aminophenol was done by the procedure reported earlier with some minor modifications [46]. The fusion of acids (1–10) and 2-aminophenol was carried out by the use of polyphosphoric acid, giving better yields (57–70%) and purity of benzoxazole derivatives (11–20).

The prototype intermediate compound 1, (1-Benzyl-5-oxopyrrolidine-3-carboxylic acid), revealed typical peaks at 1627 cm−1 (carbonyl of acid), 1734 cm−1 (carbonyl of pyrrolidin-2-one), and 3241 cm−1 (O-H of acid) in IR spectrum. In 1H-NMR spectrum of compound 1, two methyl protons were found resonating as singlet at δ 3.62. The multiplets resonating at δ 2.62–2.76 indicated two COCH2 protons, and at δ 4.29–4.44 they indicated two NCH2 and one CH proton of 2-pyrrolidinone ring. Five aromatic protons of the benzyl ring were found resonating at δ 7.20–7.36 as a multiplet. The typical singlet was assigned to COOH proton at δ 11.35 and was found to be D2O exchangeable. The M+ (molecular ion) peak of compound 11 was found at 292.14, validating its successful synthesis.
Figure 2. Design of novel pyrrolidin-2-one linked benzoxazole MAGL inhibitors. (A) Binding pattern of reported pyrrolidin-2-one MAGL inhibitors; ZINC12863377 [42], compound 25 [45] and (R)-3t [36]. (B) Designed compounds (11–20).

Figure 3. Scheme for synthesis of the intermediates (1–10) and target compounds (11–20). (a) Reflux in water (Method 1); (b) Fusion at 130–140 °C (Method 2); (c) 2-aminophenol, polyphosphoric acid, 150–160 °C, sodium carbonate.
2.2. Human MAGL Assay

The assay was executed by Cayman’s assay kit by the reported procedure [29]. All the ten compounds (11–20) were screened for hMAGL inhibitory potential. The substituted phenyl derivatives (13–20) were established to reduce the MAGL activity at 100 µM concentration below 50%. Compound 19 (4-NO2 derivative) and compound 20 (4-SO2NH2 derivative) were the most potent, with an IC50 of 8.4 and 7.6 nM, correspondingly. The structure–function relationship of benzoxazole derivatives is displayed hMAGL inhibitory activity as follows: 4-SO2NH2 > 4-NO2 > 3-Cl,4-F > 4-OCH3 > 4-Cl > 4-OH > 4-CH3 > 2-CH3 > phenyl/benzyl. The IC50 of standard controls, selective MAGL inhibitors, CAY10499 (IC50 = 415 nM), and for JZL184 (IC50 = 10 nM) were comparable to the reported values [29].

The outcomes of the experiments are presented in Table 1.

Table 1. In vitro hMAGL and hFAAH inhibition assay of the synthesized compounds (11–20).

| Compound | R          | hMAGL; IC50 | hFAAH; IC50 |
|----------|------------|-------------|-------------|
| 11       | –          | >100 µM     | ND          |
| 12       | –          | >100 µM     | ND          |
| 13       | H2C        | 85 ± 1.5 µM | ND          |
| 14       | –          | 72 ± 2.1 µM | ND          |
| 15       | –          | 65 ± 3.2 nM | 28 ± 1.8 µM |
| 16       | –          | 34 ± 1.7 nM | 25 ± 2.3 µM |
| 17       | –          | 62 ± 2.7 µM | ND          |
| 18       | –          | 42 ± 1.5 nM | 37 ± 2.2 µM |
| 19       | –          | 8.4 ± 1.9 nM| 55 ± 2.7 µM |
| 20       | –          | 7.6 ± 0.8 nM| 68 ± 2.1 µM |
| CAY10499 | –          | 415 ± 3.2 nM| –           |
| JZL184   | –          | 10 ± 0.8 nM | –           |
| URB597   | –          | 5 ± 0.6 nM  | –           |

ND: Not determined; CAY10499, JZL184 and URB597 (standard control); IC50 values were calculated from GraphPad Prism (ver. 8.0.2). Results are expressed as mean ± SEM (n = 3).
2.3. Human FAAH Assay

Derivatives having IC\textsubscript{50} in nanomolar range (compound 15, 16, 18, 19, and 20) were nominated for further screening against FAAH, an allied hydrolase of MAGL [47]. They displayed micromolar potency, with an IC\textsubscript{50} value ranging from 25 to 68 \( \mu \)M against FAAH. The benzoxazole derivatives having 4-NO\textsubscript{2} phenyl (19) and 4-SO\textsubscript{2}NH\textsubscript{2} phenyl (20), with an FAAH IC\textsubscript{50} value greater than 50 \( \mu \)M, were considered selective MAGL inhibitors. The IC\textsubscript{50} of standard control, URB597 (selective FAAH inhibitor), was 5 nM, equivalent to the value reported (IC\textsubscript{50} = 4.6 nM) [48]. The outcomes of the experiments are presented in Table 1.

2.4. Molecular Docking Study

Most active and selective compounds identified by MAGL and FAAH inhibition assay (19 and 20), were docked at the catalytic center of MAGL, in order to get an insight of their binding pattern, with the help of XP Glide docking using Maestro (Schrodinger). Compounds 19 and 20, showed comparable docking scores of \(-9.87\) and \(-9.83\), respectively. The binding of compounds 19 and 20 in the active site of MAGL revealed that the carbonyl group of pyrrolidinone is located exactly in the oxyanion hole and stabilized by three hydrogen bonds (~2Å) with alanine 51, serine 122, and methionine 123. Serine 122 is one of the critical amino acid residues of the catalytic triad of MAGL. The benzoxazole moiety is found to be positioned in the amphiphilic pouch, having \(\pi\)-\(\pi\) stacking contact with the amino acid Tyr194. The 4-NO\textsubscript{2} (19) and 4-SO\textsubscript{2}NH\textsubscript{2} (20) phenyl ring of the ligands were involved in hydrophobic (van der Waals) attractions with the amino acids, leucine 148, 213, and 241. In addition, the 3D and 2D binding pattern of compounds 19 and 20 in the catalytic location of MAGL is depicted in Figure 4.

![Figure 4](image-url)

**Figure 4.** Glide XP docking; 3D and 2D representation of the binding pattern of compound 19 (A), and 20 (B) in the catalytic site of MAGL. The picture (2D and 3D) for the docked complexes were obtained from Discovery studio visualizer 2020. The hydrophobicity was calculated from the default option available.
2.5. Pharmacokinetic and Physicochemical Characteristics

To investigate the potential of the identified derivatives (19 and 20) to cross the selectively permeable membranes of hematoencephalic barrier (BBB), to develop orally active CNS drugs, their pharmacokinetic and physicochemical features were computed by QikProp (ADMET predictor) of Schrodinger. Guidelines, concerning the validation and optimization of orally active CNS compounds, were developed by Ghose et al., by analyzing 35 characteristic features of orally bioavailable 317 CNS and 626 non-CNS drugs [49]. This guideline states that in order to design high-quality CNS drugs, the molecule must qualify by the following parameters: TPSA less than 76 Å² (ideally 25–60 Å²), number of N atoms between 1–2, comprising 1 aliphatic amine, 2–4 side chains on/outside rings, number of polar H atoms < 3 (ideally 0–1), SASA 460–580 Å², molecular volume 740–970 Å³, and must have +ve QikProp CNS property. Remarkably, most of the properties of compounds 19 and 20, computed by QikProp, were found to be in the qualifying range as per the proposed guideline (Table 2). The properties of compound 19 were found to be within the qualifying range except dipole moment. For compound 20, 5 out of 35 properties is just slightly above the upper qualifying limit. Most importantly, qualifying limits for CNS active drugs in terms of TPSA is from 3.8 to 109, and the calculated TPSA for compound 20 was found to be 119.08. Therefore, the designing of more potent MAGL inhibitors having physicochemical and pharmacokinetic properties within the preferred CNS limits is required.

Table 2. Physicochemical and pharmacokinetic properties of compounds 19 and 20, predicted by QikProp, Schrodinger, for CNS activity.

| S. No. | Property | Description | Range of Properties in CNS Drugs | Compound 19 | Compound 20 |
|--------|----------|-------------|---------------------------------|-------------|-------------|
| 1      | #stars   | drug likeness penalty; the higher the value, the less drug-like the molecule | 0 0 0 3 | 0 0 |
| 2      | #amine   | no. of basic amines | 0 1 1 2 | 0 0 |
| 3      | #amidine | no. of amidines groups | 0 0 0 0 | 0 0 |
| 4      | #acid    | no. of carboxylic acid groups | 0 0 0 0 | 0 0 |
| 5      | #amide   | no. of amides groups | 0 0 0 1 | 0 0 |
| 6      | #rotor   | no. of rotatable bonds (without CX3, alkene, amide, small ring) | 0 3 6 8 | 1 2 |
| 7      | CNS      | a qualitative CNS activity parameter | −2 0 1 2 | −2 −2 |
| 8      | dipole   | computed dipole moment | 0.67 1.1 3.9 8.9 | 9.47 10.22 |
| 9      | SASA     | solvent accessible surface area | 348 487 620 798 | 584.85 617.51 |
| 10     | FOSA     | SASA on saturated carbon and attached hydrogen | 16 178 314 464 | 91.99 91.96 |
| 11     | FISA     | SASA on N, O, and H attached to heteroatoms | 0 0 64 176 | 167.04 210.06 |
| 12     | PISA     | π component of SASA | 0 160 292 343 | 325.81 313.57 |
| 13     | WPSA     | weakly polar component of the SASA (halogens, P, and S) | 0 0 0 126 | 0 1.94 |
| 14     | volume   | estimated no. of hydrogen bonds that would be donated to the solvent water | 492 830 1104 1388 | 1002.52 1065.46 |
| 15     | donorHB  | estimated no. of hydrogen bonds that would be accepted from the solvent water | 0 0 1 3 | 0 2 |
| 16     | acceptHB | estimated no. of hydrogen bonds that would be accepted from the solvent water | 1 2.8 5.2 8.3 | 6 9.5 |
| 17     | glob     | a globularity descriptor (1 for a sphere) | 0.77 0.82 0.88 0.93 | 0.82 0.81 |
| 18     | QPpolrz  | predicted polarizability (Å³) | 14 28 38 49 | 36.43 38.19 |
| 19     | QPlogPo/w | octanol–water logP | −0.16 2.5 4.7 6.0 | 2.13 0.90 |
| 20     | QPlogS   | solubility in log(moles/liter) | −6.5 −4.6 −2.5 −0.42 | −3.99 −3.99 |
| 21     | CIPQlogS | conformation-independent solubility | −6.3 −4.2 −2.3 0.36 | −4.16 −3.77 |
| 22     | QPPCaco  | apparent Caco-2 cell permeability | 0 0 810 3269 | 258.09 100.92 |
| 23     | QPlogBB  | brain/blood partition coefficient | −1.2 −0.06 0.75 1.2 | −1.12 −1.65 |
Table 2. Cont.

| S. No. | Property Description                  | Range of Properties in CNS Drugs | Compound 19 | Compound 20 |
|--------|---------------------------------------|----------------------------------|-------------|-------------|
| 24     | QPPMDCK predicted apparent MDCK cell permeability (nm/s) | 0 | 0 | 634 | 5899 | 114.43 | 42.50 |
| 25     | QPlogKhsa prediction of binding to human serum albumin | −1 | 0.04 | 0.78 | 1.04 | −0.11 | −0.34 |
| 26     | Human oral absorption                  | 2 | 3 | 3 | 3 | 3 | 3 |
| 27     | Percent of human oral absorption       | 61 | 95 | 100 | 100 | 82.63 | 68.11 |
| 28     | van der Waals surface area of polar nitrogen and oxygen atoms | 3.8 | 12 | 54 | 109 | 98.65 | 119.08 |
| 29     | #NandO no. of N and O atoms           | 1 | 2 | 4 | 7 | 7 | 7 |
| 30     | RuleOfFive no. of violations of Lipinski’s rule of five | 0 | 0 | 0 | 1 | 0 | 0 |
| 31     | RuleOfThree no. of violations of Jorgensen’s rule of three | 0 | 0 | 0 | 1 | 0 | 0 |
| 32     | #in34 no. of atoms in three- or four-membered rings | 0 | 0 | 0 | 0 | 0 | 0 |
| 33     | #in56 no. of atoms in five- or six-membered rings | 5 | 11 | 17 | 24 | 20 | 20 |
| 34     | #noncon no. of atoms not able to form conjugation in nonaromatic rings | 0 | 0 | 4 | 10 | 3 | 3 |
| 35     | #nonH no. of non-H atoms               | 8 | 19 | 25 | 30 | 24 | 25 |

Abbreviations: QL, qualifying lower limit; PL, preferred lower limit; QU, qualifying upper limit; PU, preferred upper limit. # QL, PL, QU and PU values for CNS drug criteria were obtained from reference [49].

2.6. In Silico Absorption and Toxicity Profile

The selected compounds (19 and 20) were evaluated for their absorption and toxicity profile by a bioinformatics tool admetSAR [50]. The results suggested that both the compounds have high blood–brain barrier (BBB) penetration properties as well as high chance of human intestinal absorption. In AMES test, compound 19 was found to be mutagenic, while 20 was non-mutagenic. Carcinogenicity test revealed that both the compounds were non-carcinogens. The LD50 values in rat were also evaluated, a compound with high value is considered as less lethal. The LD50 for compounds 19 and 20 were found to be 2.30 and 2.21 mol/kg, respectively. Overall, compound 20 has better toxicity profile as compared to compound 19 (Table 3).

Table 3. In-silico absorption and toxicity profile of compounds 19 and 20 obtained from admetSAR server [50].

| Compound | BBB | HIA | HOB | AMES test | Carcinogenicity | Rat Acute Toxicity (LD50, mol/kg) |
|----------|-----|-----|-----|-----------|-----------------|----------------------------------|
| 19       | Yes | Yes | Yes | Mutagenic | Non-carcinogen  | 2.30                             |
| 20       | Yes | Yes | Yes | Non-Mutagenic | Non-carcinogen | 2.21                             |

BBB: blood–brain barrier; HIA: human intestinal permeability; HOB: human oral bioavailability; AMES test is to detect a probable mutagen; carcinogenicity estimates the cancer causing ability of a molecule; LD50: lethal dose which could kill 50% of the population of the organism (rat) on which it is being tested.

2.7. Analgesic Activity

The formalin-induced analgesic test is an extensively acknowledged animal nociception model. In order to evaluate both central and peripheral effects of the compound (20), formalin-induced nociception model was selected for analgesic activity. The formalin induced behavioral response comprises two typical phases, stage I and II. Stage I persists up to five minutes after formalin injection and is characterized by acute pain with vigorous licking and biting of the injected site. Stage I consists the action formalin on afferent C-fiber nociceptors. While Stage II starts 10–30 min after formalin injection and persists till 60 min, characterized by periodic licking and biting of the injected site. Stage II imitates
the action of central sensitization of the spinal dorsal horn neurons [51,52]. Compound 20 was selected for analgesic activity due to its higher potency (IC$_{50}$ 7.6 nM). Compound 20 (suspensions prepared with 0.5% CMC) were administered per oral (p.o, in doses of 5, 10, 30, and 50 mg/kg body weight, 4 h prior to the formalin injection. Gabapentin (GBP), (dissolved in 0.9% normal saline), was chosen as positive control (reference drug) and administered intraperitoneal (i.p) in 100 mg/kg dose. GBP exhibited little analgesic effects in Stage I (acute nociception), in comparison to the control (0.5% CMC). Though, in Stage II, it displayed significant reduction of paw licking and biting, endorsing GBP central effects. However, compound 20, reduced the pain response significantly both in acute (Stage I) and late (Stage II) phases, in a dose-dependent manner. They significantly demonstrated the reduction in pain response, having better potency than the positive control GBP at 30 mg/kg. The duration (in seconds) of paw licking and paw biting throughout Stage I and II is provided in Figure 5.

![Figure 5. Formalin-induced analgesic test; a dose of the test compound 20 (5, 10, 30, and 50 mg/kg, p.o, suspended in 0.5% CMC) was administered 4 h before formalin injection (50 µL, 2.5%). Reference drug, gabapentin (100 mg/kg, i.p, dissolved in 0.9% normal saline) was injected 30 min before formalin injection. Total paw licking and biting duration, Stage I (white bar, 0–5 min) and stage II (black bar, 10–30 min) was recorded as a measure of pain behavior. Data is represented as mean ± SEM from a group of 10 animals. * p < 0.05, ** p < 0.01, p > 0.05 (NS, nonsignificant) vs. control (vehicle). GBP: Gabapentin; D5, D10, D30, and D50 are the dose concentrations of test compound 20.](image)

2.8. Anticancer Activity

Compounds 19 and 20 were supplied to National Cancer Institute (USA), for sulforhodamine B (SRB) assay and anticancer screening [53,54]. Single-dose (10 µM) assay results for compounds 19 and 20 were provided as a mean of percent growth (% G) and growth inhibition (% GI) against 60 cell lines of nine types of cancers and are tabulated in Table 4. Derivatives 19 (NSC: 778839) and 20 (NSC: 778842) were found to have good anticancer activity towards SNB-75 cell line of CNS cancer, having % growth inhibition (% GI) of 35.49 and 31.88, respectively. Compound 20 showed 22.22 and 18.03% GI of HOP-92 and HOP-62 cell lines of non-small cell lung cancer, respectively. Both the compounds 19 and 20 were also found active on UO-31 renal cancer cell line with % GI of 21.18 and 29.95, respectively. Compound 19 showed % GI of 19.99 on T-47D, while compound 20 showed % GI of 19.89 on MDA-MB-231/ATCC cell lines of breast cancer.
Table 4. In vitro anticancer screening of compound 19 and 20, against NCI60 cell lines at 10 µM concentration.

| Panel                      | Cell Line    | % G (NSC: 778839) | % GI | % G (NSC: 778842) | % GI |
|----------------------------|--------------|-------------------|------|-------------------|------|
|                            | CCRF-CEM     | 93.58             | 6.42 | 94.48             | 5.52 |
|                            | HL-60(TB)    | 93.09             | 6.91 | 96.03             | 3.98 |
|                            | K-562        | 98.41             | 1.59 | 98.83             | 1.17 |
|                            | MOLT-4       | 93.64             | 6.36 | 92.47             | 7.53 |
|                            | RPMI-8226    | 101.28            | -1.28| 103.53            | -3.53|
|                            | SR           | 88.92             | 11.08| 93.20             | 6.80 |
| Leukemia                   | A549/ATCC    | 100.60            | -0.60| 95.28             | 4.72 |
|                            | HOP-62       | 85.04             | 14.96| 81.97             | 18.03|
|                            | HOP-92       | 104.72            | -4.72| 77.78             | 22.22|
|                            | NCI-H226     | 98.63             | 1.37 | 92.75             | 7.25 |
|                            | NCI-H23      | 93.07             | 6.93 | 92.19             | 7.81 |
|                            | NCI-H322M    | 94.18             | 5.82 | 99.10             | 0.90 |
|                            | NCI-H460     | 102.33            | -2.33| 104.20            | -4.20|
| Non-Small Cell Lung Cancer | COLO 205     | 103.79            | -3.79| 104.05            | -4.05|
|                            | HCC-2998     | 102.05            | -2.05| 100.24            | -0.24|
|                            | HCT-116      | 102.14            | -2.14| 95.78             | -4.22|
|                            | HCT-15       | 98.10             | 1.9  | 101.16            | -1.16|
|                            | HT29         | 99.25             | 0.75 | 103.23            | -3.23|
|                            | KM12         | 105.43            | -5.43| 101.16            | -1.16|
|                            | SW-620       | 102.52            | -2.52| 102.95            | -2.95|
| Colon Cancer               | SF-288       | 91.85             | 8.15 | 87.43             | 12.57|
|                            | SF-295       | 98.21             | 1.79 | 93.71             | 6.29 |
|                            | SF-539       | 95.59             | 4.41 | 87.58             | 12.42|
|                            | SNB-19       | 99.29             | 0.71 | 97.02             | 2.98 |
|                            | SNB-75       | 64.51             | 35.49| 68.12             | 31.88|
|                            | U251         | 100.97            | -0.97| 95.21             | 4.79 |
| CNS Cancer                 | LOX IMVI     | 89.06             | 10.94| 92.99             | 7.01 |
|                            | MALME-3M     | 88.53             | 11.47| 93.29             | 6.71 |
|                            | M14          | 101.37            | -1.37| 98.58             | 1.42 |
|                            | MDA-MB-435   | 95.05             | 4.95 | 100.55            | -0.55|
|                            | SK-MEL-2     | 102.31            | -2.31| 111.54            | -11.54|
|                            | SK-MEL-28    | 111.25            | -11.25| 101.79            | -1.79|
|                            | SK-MEL-5     | 98.72             | 1.28 | 98.82             | -1.18|
|                            | UACC-257     | 106.92            | -6.92| 110.78            | -10.78|
|                            | UACC-62      | 97.59             | 2.41 | 92.78             | 7.22 |
| Melanoma                   | IGROV1       | 104.14            | -4.14| 101.63            | -1.63|
|                            | OVCAR-3      | 98.56             | 1.44 | 98.53             | 1.47 |
|                            | OVCAR-4      | 106.27            | -6.27| 99.47             | 0.53 |
|                            | OVCAR-5      | 98.30             | 1.70 | 92.35             | 7.65 |
|                            | OVCAR-8      | 101.95            | -1.95| 97.49             | 2.51 |
|                            | NCI/ADR-RES  | 98.37             | 1.63 | 101.45            | -1.45|
|                            | SK-OV-3      | 88.34             | 11.66| 94.77             | 5.23 |
| Ovarian Cancer             | 786-0        | 104.06            | -4.06| 98.99             | 1.01 |
|                            | A498         | 113.46            | -13.46| 113.94            | -13.94|
|                            | ACHN         | 91.70             | 8.3  | 89.48             | 10.52|
|                            | Caki-1       | 97.19             | 2.81 | 92.35             | 7.65 |
|                            | SN12C        | 97.21             | 2.79 | 95.50             | 4.50 |
|                            | TK-10        | 110.14            | -10.14| 114.82            | -14.82|
|                            | UO-31        | 78.82             | 21.18| 70.05             | 29.95|
| Renal Cancer               | PC-3         | 91.14             | 8.86 | 88.11             | 11.89|
|                            | DU-145       | 110.09            | -10.09| 111.62            | -11.62|
| Prostate Cancer            | MCF7         | 99.19             | 0.81 | 92.32             | 7.68 |
|                            | MDA-MB-231/ATCC | 88.41      | 11.59| 80.11             | 19.89|
|                            | HS 578T      | 101.81            | -1.81| 104.19            | -4.19|
|                            | T-47D        | 80.01             | 19.99| 83.24             | 16.24|
|                            | MDA-MB-468   | 98.03             | 1.97 | 100.34            | -0.34|
3. Discussion

Ten benzoxazole clubbed 2-pyrrolidinone derivatives (11–20) as the inhibitors of monoacylglycerol lipase were designed on the criteria fulfilling the structural requirements and on the basis of previously reported inhibitors [36,42–45]. The designed, synthesized, and characterized compounds (11–20) were screened against monoacylglycerol lipase (MAGL) in order to find potential inhibitors. The substituted phenyl derivatives (13–20) were established to reduce the MAGL activity at 100 µM concentration below 50%. Compound 19 (4-NO₂ derivative) and compound 20 (4-SO₂NH₂ derivative) were the most potent, with IC₅₀ of 8.4 and 7.6 nM, correspondingly. The benzoxazole derivatives having 4-NO₂ phenyl (19) and 4-SO₂NH₂ phenyl (20), with an FAAH IC₅₀ value greater than 50 µM, were considered selective MAGL inhibitors. In molecular docking studies, compounds 19 and 20 showed comparable docking scores of −9.87 and −9.83, respectively. The binding of compounds 19 and 20 in the active site of MAGL revealed that the carbonyl group of pyrrolidinone is located exactly in the oxyanion hole and stabilized by three hydrogen bonds (~2 Å) with alanine 51, serine 122, and methionine 123. Serine 122 is one of the critical amino acid residues of the catalytic triad of MAGL. The benzoxazole moiety is found to be positioned in the amphiphilic pouch, having π-π stacking contacts with the amino acid tyrosine 194. The 4-NO₂ phenyl (19) and 4-SO₂NH₂ phenyl (20) part of the ligand was engaged in hydrophobic (Van der Waals) attractions with the amino acids leucine 148, 213, and 241. The binding patterns of compounds 19 and 20 in the catalytic site of MAGL were found to be similar as those of the reported inhibitors bound crystal structures [32,36,39,41]. Remarkably, the physiochemical and pharmacokinetic properties of compounds 19 and 20 computed by QikProp were found to be in the qualifying range as per the proposed guideline for good orally bioactive CNS drugs. Moreover, compound 20 showed better toxicity profile than compound 19, as predicted by admetSAR [50]. In formalin-induced analgesic test, compound 20 reduced the pain response significantly both in acute (stage I) and late (stage II) phases in a dose-dependent manner. It significantly demonstrated the reduction in pain response, having better potency than the positive control GBP, at the dose of 30 mg/kg. Moreover, in one dose (10 µM), anticancer screening by SRB assay, compounds 19 (NSC: 778839) and 20 (NSC: 778842) were found to have good anticancer activity towards SNB-75 cell line of CNS cancer, having % growth inhibition (% GI) of 35.49 and 31.88, respectively. Therefore, the present work concluded that compound 20 is the potential lead compounds that can be further manipulated at points 1 and 4 of the 2-pyrrolidinone moiety for the discovery and development of more selective and potent inhibitors of MAGL for neuropathic pain and CNS disorders including cancers.

4. Experimental

4.1. Chemistry

Reagents and solvents were procured from Merck Ltd. (New Delhi, India) and Sigma-Aldrich Ltd. (New Delhi, India). Progress and completion of the reactions was checked by thin-layer chromatography (TLC). Melting points of the derivatives were determined by open tube capillary method and uncorrected. Elemental analysis data were obtained from CHNOS elemental analyzer (Vario EL III, Elementar Analysensysteme GmbH, Langenselbold, Germany). Shimadzu FT-IR spectrometer (Shimadzu Analytical Pvt. Ltd., New Delhi, India) was used for recording IR spectrum (4000–400 cm⁻¹), by preparing KBr pellets. ¹H-NMR spectrum of the derivatives were obtained from Bruker 300 MHz NMR instrument (Bruker Avance AV-III type, Billerica, MA, USA) using CDCl₃ or DMSO-d₄ as solvent. ¹H-NMR spectra of compounds 11–20 can be found in the Supplementary Material. Molecular mass (m/z) of the derivatives were obtained by UPLC-MS (Q-TOF-ESI) (Waters Corp., Milford, MA, USA).
4.1.1. Synthesis of 1-(Aryl Substituted)-5-Oxopyrrolidine-3-Carboxylic Acids (1–10)

Method-1 (for compound 1)

Equimolar amount of benzylamine (50 mmol) and itaconic acid (50 mmol, 6.5 g) in 50 mL of triple distilled water was refluxed for about 45–60 min. The contents were then chilled, filtered, and washed with cold water. The obtained solid was dissolved in minimum quantity of aq. NaOH (10%). After treatment with activated charcoal, the solution was filtered and acidicified with dil. HCl in order to obtain the precipitate. The filtered solid was washed with cold water, dried, and purified by recrystallization from ethanol/water mixture.

1-Benzyl-5-Oxopyrrolidine-3-Carboxylic Acid (1), white solid; yield: 75%; m.p. 142–145°C; IR: 1411 (C-N), 1562 (C=C), 1598 (C=N), 1697 (C=O), 3077 (Ar C-H); ESI-MS (m/z): 219.12 [M+H]+; Anal. calcd. For C18H16NO2: C, 73.95; H, 5.52; N, 9.58. Found: C, 74.21; H, 5.82; N, 9.82.

Intermediate compounds (2–10) were synthesized as per the procedure reported in our previous publication [45].

4.1.2. Synthesis of 4-(Benzoazolyl)-1-(Aryl Substituted)Pyrrolidin-2-Ones (11–20)

Appropriate 1-aryl substituted)-5-oxopyrrolidine-3-carboxylic acids (20 mmol), 2-aminothiphene (20 mmol, 2.18 g) and polyphosphoric acid (20 g) in an RBF were heated to 150–160°C and stirred for 2–3 h. The content of the RBF (round bottom flask) was cooled at RT; 5% NaCO3 (25 mL) was added and heated for 10 min. The content was chilled and transferred in a flask having 100 mL of water, and stirred at RT for 15 min. The filtered solid was washed three times with water (50 mL), dried, and purified by recrystallization with ethanol. The derivatives were then purified by chromatography using ethylacetate:hexane (1:4) as solvent.

4-(Benz[d]oxazol-2-yl)-1-benzylpyrrolidin-2-one (11), pale-yellow solid; yield: 57%; m.p. 160–162°C; IR: 1370 (C=C), 1555 (C=C), 1621 (C=O), 1703 (C=O), 3077 (Ar C-H); 1H-NMR (DMSO-d6) δ (ppm): 2.60–2.77 (m, 2H, COCH=), 3.13–3.18 (m, 1H, CHpyrr), 3.81–3.86 (m, 2H, NCH2 and CHpyrr), 7.20–7.36 (m, 5H, Ar-H), 11.35 (s, 1H, COOH, D2O exchangeable); ESI-MS (m/z): 292.14 [M]+; 293.14 [M+H]+; Anal. calcd. for C17H16NO2: C, 73.79; H, 5.57; N, 9.58. Found: C, 74.16; H, 5.70; N, 9.82.

4-(Benz[d]oxazol-2-yl)-1-phenylpyrrolidin-2-one (12), pale-yellow solid; yield: 67%; m.p. 148–150°C; IR: 1395 (C-N), 1552 (C=C), 1615 (C=O), 1698 (C=O), 3070 (Ar C-H); 1H-NMR (DMSO-d6) δ (ppm): 2.86–3.20 (m, 2H, COCH2), 4.16–4.40 (m, 3H, NCH2 and CHpyrr), 7.16–8.18 (m, 9H, Ar-H); ESI-MS (m/z): 278.13 [M]+, 279.13 [M+H]+; Anal. calcd. for C17H16NO2: C, 73.73; H, 5.07; N, 10.07. Found: C, 73.60; H, 5.18; N, 10.33.

4-(Benz[d]oxazol-2-yl)-1-(o-tolyl)pyrrolidin-2-one (13), pale-yellow solid; yield: 68%; m.p. 162–164°C; IR: 1404 (C-N), 1568 (C=C), 1607 (C=O), 1691 (C=O), 3079 (Ar C-H); 1H-NMR (DMSO-d6) δ (ppm): 2.18 (s, 3H, CH3), 2.88–3.17 (m, 2H, COCH2), 4.12–4.18 (m, 1H, CHpyrr), 4.26–4.39 (m, 2H, NCH2), 7.18–7.21 (m, 2H, H-5-pyrrol) and H-6(pyrrol)), 7.36–7.54 (m, 4H, H-5-benzoxazole), H-benzoxazole, H-3(pyrrol) and H-4(pyrrol)), 7.98–8.01 (d, 1H, H-7-benzoxazole, J = 8.1 Hz), 8.09–8.12 (d, 1H, H-4-benzoxazole, J = 7.8 Hz); ESI-MS (m/z): 292.13 [M]+, 293.13 [M+H]+; Anal. calcd. for C18H16NO2: C, 73.95; H, 5.52; N, 9.58. Found: C, 74.21; H, 5.82; N, 9.77.

4-(Benz[d]oxazol-2-yl)-1-(p-tolyl)pyrrolidin-2-one (14), pale-yellow solid; yield: 67%; m.p. 164–166°C; IR: 1411 (C-N), 1562 (C=C), 1598 (C=O), 1697 (C=O), 3077 (Ar C-H); 1H-NMR (DMSO-d6) δ (ppm): 2.28 (s, 3H, CH3), 2.94–3.13 (m, 2H, COCH2), 4.13–4.35 (m, 3H, NCH2 and CHpyrr), 7.17–7.20 (d, 2H, H-3(pyrrol) and H-5(pyrrol), J = 8.4 Hz), 7.34–7.42 (m,
4-(Benzo[d]oxazol-2-yl)-1-(4-nitrophenyl)pyrrolidin-2-one (15), pale-yellow solid; yield: 70%; m.p. 174–176 °C; IR: 758 (C=Cl), 1396 (C=N), 1568 (C=C), 1612 (C=C), 1689 (C=O), 3086 (Ar C-H); 1H-NMR (DMSO-d6) δ (ppm): 3.01–3.09 (m, 2H, COCH3), 4.20-4.33 (m, 3H, NCH2 and CHpyrr), 7.35–7.43 (m, 4H, Ar-H), 7.68–7.72 (t, 4H, Ar-H, J = 6.3 Hz); ESI-MS (m/z): 312.11 [M]+, 314.10 [M + 2]+; Anal. calcd. for C18H19N2O2: C, 73.95; H, 5.52; N, 9.58. Found: C, 74.18; H, 5.78; N, 9.80.

4-(Benzo[d]oxazol-2-yl)-1-(4-chlorophenyl)pyrrolidin-2-one (16), pale-yellow solid; yield: 68%; m.p. 187–189 °C; IR: 765 (C=Cl), 1388 (C=N), 1545 (C=C), 1613 (C=C), 1690 (C=O), 3098 (Ar C-H); 1H-NMR (DMSO-d6) δ (ppm): 2.91–3.19 (m, 2H, COCH3), 4.13–4.21 (m, 1H, CHpyrr), 4.25–4.41 (m, 2H, NCH2), 7.41–7.60 (m, 5H, Ar-H), 8.05–8.08 (d, 1H, H-7 benzoxazole, J = 8.1 Hz), 8.11–8.14 (d, 1H, H-4 benzoxazole, J = 8.1 Hz); ESI-MS (m/z): 330.10 [M]+, 332.09 [M + 2]+; Anal. calcd. for C17H12ClF2N2O: C, 65.73; H, 3.66; N, 8.47. Found: C, 62.07; H, 3.85; N, 8.61.

4-(Benzo[d]oxazol-2-yl)-1-(4-hydroxyphenyl)pyrrolidin-2-one (17), pale-yellow solid; yield: 62%; m.p. 242–245 °C; IR: 1407 (C=N), 1535 (C=C), 1597 (C=N), 1698 (C=O), 3092 (Ar C-H), 3410 (O-H); 1H-NMR (DMSO-d6) δ (ppm): 2.91–3.20 (m, 2H, COCH3), 4.12–4.23 (m, 1H, CHpyrr), 4.25–4.39 (m, 2H, NCH2), 5.64 (bs, 1H, OH, D2O exchangeable), 7.41–7.60 (m, 4H, Ar-H phenyl), 7.72–7.77 (t, 2H, H-5 benzoxazole and H-6 benzoxazole, J = 7.5 Hz), 7.96–7.99 (d, 1H, H-7 benzoxazole, J = 8.4 Hz), 8.08–8.10 (d, 1H, H-4 benzoxazole, J = 8.4 Hz); ESI-MS (m/z): 294.12 [M]+, 295.12 [M + H]+; Anal. calcd. for C17H12ClF2N2O: C, 65.73; H, 3.66; N, 8.47. Found: C, 62.07; H, 3.85; N, 8.61.

4-(Benzo[d]oxazol-2-yl)-1-(3-chloro-4-fluorophenyl)pyrrolidin-2-one (18), pale-yellow solid; yield: 66%; m.p. 177–179 °C; IR: 1402 (C=N), 1546 (C=C), 1600 (C=C), 1690 (C=O), 3081 (Ar C-H); 1H-NMR (DMSO-d6) δ (ppm): 2.88–3.18 (m, 2H, COCH3), 3.67 (s, 3H, OCH3), 4.08–4.16 (m, 1H, CHpyrr), 4.22–4.39 (m, 2H, NCH2), 7.16–7.19 (d, 2H, H-2 phenyl and H-6 phenyl, J = 8.4 Hz), 7.42–7.58 (m, 4H, H-5 benzoxazole, H-6 benzoxazole, H-3 phenyl and H-5 phenyl), 7.95–7.98 (d, 1H, H-7 benzoxazole, J = 8.4 Hz), 8.09–8.12 (d, 1H, H-4 benzoxazole, J = 8.4 Hz); ESI-MS (m/z): 308.13 [M]+, 309.13 [M + H]+; Anal. calcd. for C18H16F2N2O: C, 70.12; H, 5.23; N, 9.09. Found: C, 70.35; H, 5.38; N, 9.18.

4-(Benzo[d]oxazol-2-yl)-1-(4-nitrophenyl)pyrrolidin-2-one (19), yellow solid; yield: 62%; m.p. 276–278 °C; IR: 1405 (C=N), 1550 (C=C), 1595 (C=N), 1695 (C=O), 3089 (Ar C-H); 1H-NMR (DMSO-d6) δ (ppm): 2.89–3.15 (m, 2H, COCH3), 4.11–4.16 (m, 1H, CHpyrr), 4.24–4.37 (m, 2H, NCH2), 6.79–6.82 (d, 2H, H-2 phenyl and H-6 phenyl, J = 9 Hz), 7.43–7.48 (t, 2H, H-5 benzoxazole, H-6 benzoxazole, J = 7.5 Hz), 8.01–8.18 (m, 4H, H-4 benzoxazole, H-7 benzoxazole, H-3 phenyl and H-5 phenyl); ESI-MS (m/z): 323.11 [M]+, 324.11 [M + H]+; Anal. calcd. for C17H15N3O4: C, 63.16; H, 4.05; N, 13.00. Found: C, 63.35; H, 4.27; N, 13.23.

4-(4-Benzoxazol-2-yl)-2-oxopyrrolidin-1-ylbenzencesufonamide (20), yellow solid; yield: 60%; m.p. 227–229 °C; IR: 1416 (C=N), 1564 (C=C), 1607 (C=N), 1704 (C=O), 3095 (Ar C-H); 1H-NMR (DMSO-d6) δ (ppm): 2.91–3.19 (m, 2H, COCH3), 4.15–4.22 (m, 1H, CHpyrr), 4.25–4.38 (m, 2H, NCH2), 5.67 (s, 2H, SO2NH2, D2O exchangeable), 7.42–7.59 (m, 4H, Ar-H), 8.09–8.16 (m, 4H, Ar-H); ESI-MS (m/z): 357.12 [M]+, 358.12 [M + H]+; Anal. calcd. for C17H15N3S2: C, 57.13; H, 4.23; N, 11.76. Found: C, 57.36; H, 4.36; N, 11.95.

4.2. Human MAGL Assay

The screening of the synthesized compounds (11–20) for their capability to reduce hMAGL activity was performed according to the information leaflet provided with Cayman’s assay kit (Cayman Chemical, Michigan, USA) by the reported method [29], as
discussed in detail in our previous publication [45]. The results were compared with standard MAGL inhibitors, CAY10499 and JZL184, and are provided in Table 1.

4.3. Human FAAH Assay

The screening of the selected compounds (15, 16, 18, 19 and 20) for their potential to inhibit hFAAH was performed according to the information leaflet provided with Cayman’s assay kit (Cayman Chemical, Ann Arbor, Michigan, USA) by the reported method [47], as discussed in detail in our previous publication [45]. The results were compared with standard FAAH inhibitor, URB597, and are provided in Table 1.

4.4. Molecular Docking Study

Glide executed on Maestro 9.4 (Schrödinger Inc., New York, NY, USA) was utilized for Glide XP docking of active compounds (19 and 20). The .pdb file of hMAGL X-ray crystal structure was downloaded from protein data bank having ID 5ZUN (crystal structure resolution 1.35 Å) for molecular docking study [36]. The protein structure was refined, optimized, and energy-minimized with the help of preparation wizard in Maestro. A docking grid of $20 \times 20 \times 20 \text{Å}$, was created around the catalytic site by defining the cocrystallized ligand. Ligand (compounds 19 and 20) structures were prepared with the help of LigPrep 2.6 with Epik 2.4 at pH 7.0 ± 2.0. The methodology was validated by docking the cocrystallized ligand with Glide XP docking protocol [55].

4.5. Physicochemical and Pharmacokinetic Characteristics

Guidelines, concerning the validation and optimization of orally active CNS compounds, were developed by Ghose et. al. by analyzing 35 characteristic features of orally bioavailable 317 CNS and 626 non-CNS drugs [49]. For computations of these properties of the selected compounds (19 and 20), QikProp 3.6 module of Schrodinger was utilized. The generated data was then matched with the qualifying range as per the suggested guideline for good orally bioactive CNS drugs.

4.6. In Silico Absorption and Toxicity Profile

The selected compounds (19 and 20) were evaluated for their absorption and toxicity profile by a bioinformatics tool admetSAR [50]. Oral bioavailability, intestinal absorption, and BBB penetration properties were calculated. AMES test for mutagenicity, carcinogenicity test, and the calculation of LD50 for both the compounds (19 and 20) were also evaluated.

4.7. Analgesic Activity

Formalin-induced analgesic test was executed by the procedure described by Coderre and Laughlin [51,52] as discussed in detail in our previous publication [45]. Male Wistar rats (180–200 g) were obtained with the permission of IAEC (proposal number 1048) from Jamia Hamdard, New Delhi, India. The results of test compound 20 and reference drug, Gabapentin (GBP), were statistically compared with the control group.

4.8. Anticancer Screening: Sulforhodamine B Assay

Compounds 19 and 20 were supplied to National Cancer Institute (Bethesda, Maryland, USA), for in vitro sulforhodamine B (SRB) assay, anticancer screening on 60 cell lines of cancers of leukemia, melanoma, and tumors of the kidney, brain, breast, lung, colon, ovary, and prostate, as per their standard protocol [53,54]. One dose anticancer results (NCI, USA) of compounds 19 and 20 are provided in the Supplementary Materials.

4.9. Statistical Analysis

The statistical study of the data was accomplished by GraphPad Prism (version 8.0.2; GraphPad Software, San Diego, CA, USA). The dose response of the test compounds was
compared with that of control, in formalin-induced analgesic test, by analysis of variance (ANOVA) followed by Dunnett’s test. Outcomes are communicated as mean ± SEM.

5. Conclusions

Ten benzoxazole clubbed 2-pyrrolidinone derivatives (11–20) as the inhibitors of MAGL were designed, synthesized, characterized, and assayed against MAGL and FAAH enzymes, in order to find potential small molecule selective MAGL inhibitor. Compounds 19 (4-NO₂ derivative) and 20 (4-SO₂NH₂ derivative) were found most potent and selective, with an IC₅₀ of 8.4 and 7.6 nM, respectively. The binding patterns of compounds 19 and 20 in the catalytic site of MAGL were found as expected and similar as those of the reported inhibitors.

The physiochemical and pharmacokinetic properties of compounds 19 and 20 were found to be almost in the qualifying range as per the proposed guideline for good orally bioactive CNS drugs. Compound 20 significantly demonstrated the reduction in pain response, having better potency than the positive control GBP, at the dose of 30 mg/kg. The present work concluded that compound 20 is the potential lead compounds that can be further studied and optimized at points 1 and 4 of the 2-pyrrolidinone moiety for the discovery and development of more selective and potent inhibitors of MAGL for neuropathic pain.

Supplementary Materials: The following are available online, ¹H-NMR spectra of compounds 11–20 and one dose anticancer results (NCI, USA) of compounds 19 and 20 is provided in the supporting information.

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Sample Availability: The samples of the compounds are available on request from the corresponding author.

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